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CHESAPEAKE BAY NUTRIENT DYNAMICS STUDY

by

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16. ABSTRACT

This study had two major objectives. The first was to fill gaps in our understanding of important biological, chemical, and physical processes occurring in the Bay. This information was required to make the best use of existing data and to develop future data needs to further understand the basic functioning of the Bay system. The second objective was to collect a synoptic data set for the entire Chesapeake Bay to be used in future modeling efforts and to establish the present condition of the main Bay at one point in time.

Field work for the study was conducted by sixteen scientists on thirteen cruises between 1 May 1980 and 1 June 1981. The synoptic nutrient study was conducted from 8 to 17 July 1980 in conjunction with a circulation study covering the entire Bay from 25 June to 29 July 1980. The process studies were performed at various times and locations dictated by the processes themselves. Subsurface transport of nutrients and phytoplankton were examined in May 1980 in the upper Bay. Sediment nutrient releases and oxygen demand were studied in eight locations in summer 1980 and spring 1981. Nitrogen and silica dynamics were examined in several locations during July, August, and September 1980. Bacterial dynamics were studied in August 1980. The results of these studies added important information to the body of knowledge about Chesapeake Bay.

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INTRODUCTION

This document reports results from a series of studies conducted on Chesapeake Bay during 1980 and 1981. The overall objective of these studies was to fill some of the gaps in our understanding of nutrient processes in this large estuarine system. To accomplish this objective a steering committee was formed with representatives of the bay community and EPA representatives. Research topics were identified and leading scientists in several areas of estuarine research were invited to participate in the program. Many did and, as a result, new information regarding biological and chemical processes influencing water quality in the bay is becoming available. In addition, this study provided opportunities to re-examine some exisitng data sets and to collect a new, bay-wide, synoptic data set for future use by managers and modelers.

The chapters of this report relate to each of the experimental studies and to information requests made by EPA after initiation of the cooperative agreement. Data from the synoptic study conducted in July 1980 has been provided to the Chesapeake Bay Program on computer tape for use in other aspects of the Program.

Chapters I and II contain specific information already provided to the Bay Program. It is included here for sake of completeness of the final report. Chapter II describes an experiment on bacterial dynamics in the estuarine portion of the York River. Bacteria have an important role in nutrient cycles so the growth rates and biomass of representatives of this group are significant new information for the bay system. Chapter III has been accepted for publication in Limnology and Oceanography. Chapter IV describes a new modification of the chlorophyll a methodology to improve recovery in marine systems. It has been submitted for publication. Chapter V discusses the first attempts in Chesapeake Bay to measure the recycling rate of ammonium nitrogen with heavy isotope dilution techniques. Chapter VI is a comprehensive study of nitrogen transformations in the water column of Chesapeake Bay during fall. Although the processes had been noted previously, this study provides the first direct measurements for nitrification and denitrification in the open water of the bay. This chapter has been submitted to Limnology and Oceanography. Chapter VII describes a model for estimating the relative contributions of nutrients from water column recycling below the pycnocline and from sediment release. This is a new application of available data analysis techniques which uses only vertical profiles of nutrients and salinity in the calculations. This chapter has been submitted to Limnology and Oceanography. Chapter VIII discusses the exchanges of nutrients between sediments and the overlying water in several locations in Chesapeake Bay. The sediments are an important source/sink for nutrients of various times of the year. This is the most complete set of measurements made to date in the bay. This chapter will be submitted to Limnology and Oceanography. Two additional chapters concerned with the silicon cycle and with subsurface nutrient and plankton transfer are not yet completed but will be forwarded when finished.

The appendix contains visual displays of some of the data collected during the synoptic study in July 1980. The distributions of extracted chlorophyll a, salinity, particulate carbon, and suspended solids are shown for 15 July. The dissolved oxygen distributions are shown for all days on which data were taken.

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CHAPTER I

Coefficients for the Biological Model of Chesapeake Bay

J.L. Taft

1. Introduction

This report was prepared as part of the cooperative agreement between the USEPA and The Johns Hopkins University. It lists ranges for coefficients to be used in the Chesapeake Bay Program biological model.

2. Model Coefficients

a. k_{mp} - Half-saturation value for inorganic phosphate. The value for k_{mp} in open waters of Chesapeake Bay fall in the range of 0.11 to 1.7 µg atom $liter^{-1}$. Values in the Potomac River also fall in this range, but were generally less than 0.5 µg atom $liter^{-1}$.

b. k^*_r - Respiration Rate. Water column respiration has a wide range in both summer and winter. Most values fall in the range of 4 to 15 mg atom O_2 mg $Chla^{-1}$ d^{-1}. One value of 32 mg atom O_2 mg Chla $^{-1}$ d $^{-1}$ was observed in the mid-bay during August.

c. k^*_r - Grazing coefficient. Previous studies in the open bay have shown that copepods graze about 5-10% of the daily production and micro-zooplankton graze a similar fraction under non-bloom conditions. Therefore, the grazing coefficient should be 0.1 to 0.2 d^{-1} based on chlorophyll a concentration.

d. r_n - Particulate nitrogen to chlorophyll a ratio. Ratios of particulate nitrogen to chlorophyll a (µg atom: µg) vary widely in Chesapeake Bay. Typical values are:

	Winter	Spring	Summer
Upper Bay	1.5:1	2:1 to 3:1	1:1 to 2:1
Mid-Bay	2:1 to 3:1	1.5:1	2:1
Lower Bay	2:1 to 3:1	1:1 to 3:1	1:1 to 3:1

e. r_p - Particulate phosphorus to chlorophyll *a* ratio. Ratios of particulate phosphorus to chlorophyll *a* (µg atom: g) also vary widely. Typical values are:

	Winter	Spring	Summer
Upper Bay	1:25	1:25 to 1:125	1:10 to 1:20
Mid-Bay	1:16	1:30 to 1:100	1:10
Lower Bay	1:12	1:10 to 1:60	1:10

f. b_o - Benthic O_2 demand. The benthic oxygen demand during summer is in the range 36 to 105 mg atom o_2 m^{-2} d^{-1} with no discernable trends in upper or mid-bay. A single lower bay station gave a value of 36 mg atom O_2 m^{-2} d^{-1}.

g. k_e - Light extinction coefficient. The extinction coefficient for the lower bay is 1.0 to 1.1 m^{-1}. For the upper bay, k_e is 1.2 to 1.3. In benthic studies, extinction coefficients in shallower areas of the bay ranged from 0.6 to 2.0 m^{-1}.

h. k^*_4 - Coiefficient for hydrolysis of organic N. This coefficient is estimated to be 0.05 to 0.1 d^{-1}.

i. k^*_5 - Coefficient of nitrification. The coefficient of nitrification has been measured in Chesapeake Bay. It was found to be

0.1 to 0.2 d^{-1} in the fall and may be that high in spring in some tributary estuaries.

j. k_{mn} - Half sautration value for N. The value of k_{mn} for ammonium should be 1 to 2 µg atom $liter^{-1}$. The k_{mn} for nitrate should be 2 to 4 µg atom $liter^{-1}$.

k. k^*_7 - Coefficient for conversion of organic P to inorganic P. The value for k^*_7 is estimated to be 0.1 d^{-1}.

l. k^*_9 - Coefficient for CBOD exodation. The coefficient for CBOD oxidation in the open bay is 0.1 to 0.2 d^{-1}. In more eutrophic tributaries it is 0.2 to 0.3 d^{-1}.

m. k_{op} - Photosynthetic quotients. The photosynthetic quotient ranges from 1.05 for cells using ammonium as N source to 1.6 for those using nitrate. Values about 1.25 are common.

n. k_{or} - Respiratory quotient. The respiratory quotient is usually near 1.0 in natural waters.

CHAPTER II

Characterization of Segment Boundaries in Chesapeake Bay

J.L. Taft

Biological and Chemical Characterization of Segment Boundaries.

Main Bay

The Chesapeake Bay has been segmented in a way which recognizes some major biological and chemical chracteristics of the estuary.

The first boundary separates Susquehanna Flats from the upper bay and lies in the region of maximum penetration of sea salt at the head of the bay. South of this region one would not expect most fresh water plankton to grow and flourish, although some may be continually brought into the area by the Susquehanna River.

The second boundary demarcates the southern limit of the turbidity maximum, a region where suspended sediment causes light limitation of phytoplankton production most of the year. This boundary also coincides with the long term summer average for the 5 $^{o}/oo$ salinity contour which is an important physiological parameter for oysters.

The third boundary marks the northern limit of deep water anoxia in Chesapeake Bay and the 10 $^{o}/oo$ salinity contour. South of the area near the Bay Bridge the water deeper than about 30' usually experiences oxygen depletion in summer which may result in anoxia and hydrogen sulfide production. When anoxia occurs, these deep waters are toxic to fish, crabs, shellfish, and other demersal and benthic animals. The anoxic layer is also rich in nutrients which may reach the surface layer by diffusion, mixing, and vertical advection. In spring the region near the bridge is the site where phytoplankton and fish larvae that travel in the deep layer from the bay mouth are brought to the surface by a combination of physical processes.

The fourth boundary was established at a narrows which marks the southern boundary of the Susquehanna River estuary. Below this point the Patuxent and Potomac Rivers enter the main bay. It lies mid-way in the area subject to summar anoxia. It is characterized by salinities of 12 to 13 ^{o}/oo in the long term summer average.

The fifth boundary stretches across the bay from Smith Point. It approximates the southern limit of summer anoxic water and the 15 ^{o}/oo salinity contour. It also nearly coincides with the Maryland-Virginia state line. Most of the deeper areas of the bay are found between boundaries four and five. As mentioned earlier, the segments between boundaries three and five experience considerable nutrient enrichment during the summer when both phosphate and ammonium are released from suspended organic material and bottom sediments. This region also exhibits high nitrite and nitrate concentrations in Fall when the ammonium accumulated in summer is oxidized by bacteria. The Smith Point boundary also approximates the region where the nitrate from the Spring freshet becomes a critical nutrient for the phytoplankton.

The sixth boundary near Wolf Trap falls on the 18 ^{o}/oo salnity contour and separates the lower bay into two regions with different circulation patterns. North of Wolf Trap the bay's density stratification results in two distinct vertical layers. Hence the deep water there moves in a net upstream flow and the surface layer flows downstream. Between Wolf Trap and the bay mouth the density distribution tends toward a cross stream gradient rather than vertical.

The seventh boundary separates Chesapeake Bay from the coastal ocean. A pronounced horizontal salinity gradient exists across the mouth which is detectable north to boundary six. Planktonic organisms and the larvae of catadromous fish are brought into the bay with high salinity ocean water. As this dense water slips below the surface near boundary six, the organisms ar entrained in the deep water and carried up the bay to grow and mature.

Boundaries eight and nine divide major regions of submerged aquatic vegetation from waters of the main bay. Although these areas have salinity similar to the adjacent bay wters, they are shallow enough to permit light penetration necessary for submerged plant growth. The plants provide shelter for many invertebrates and small fish which contribute to the bay's natural richness.

Boundaries have been drawn across the mouths of the principle tributaries. These sub-estuaries are sources of fresh water, sediment, nutrients, and phytoplankton seed populations which may grow to bloom concentrations in the main bay.

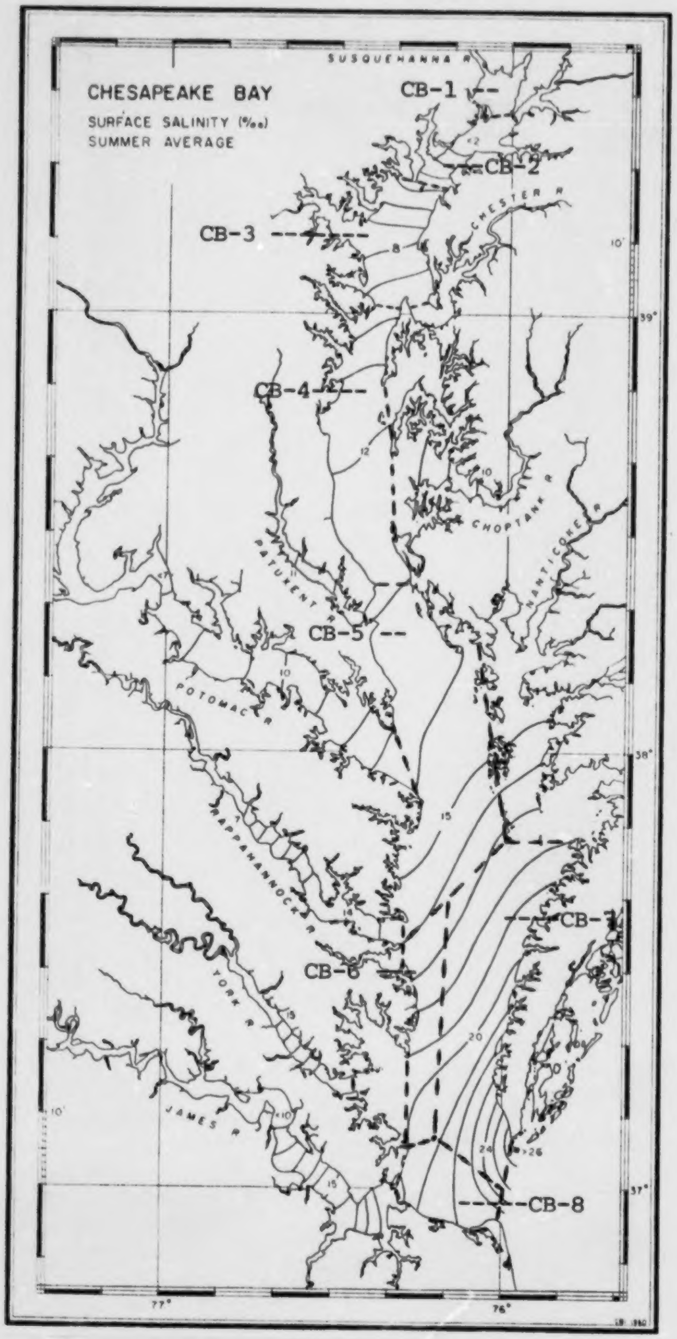

Figure 1. Map of Chesapeake Bay showing summer surface isohalines and segment boundaries for the main portion of the Bay.

CHAPTER III

Bacterial Biomass and Production During Spring Tidal Destratification in the York River, Virginia Estuary

H.W. Ducklow

ABSTRACT

Bacterial abundance, biomass, and ^{3}H-thymidine incorporation rates were studied during spring tidal destratification of the York River, Virginia estuary. In this system, monthly high spring tides cause destratification of the moderately stratified estuary, which oscillates between stratified and vertically homogeneous conditions on a time scale of 1-10 days. Bacterial abundance and carbon biomass ranged $1\text{-}8 \times 10^9$ cells 1^{-1} and 20-100 μgC 1^{-1}. Thymidine incorporation into cold TCA insoluble fractions ranged from 1-10 nano-moles 1^{-1} d^{-1} and bacterial carbon production rates and specific growth rates were 7-75 μgC $1^{-1}d^{-1}$ and 0.2 - 1.1 d^{-1}, respectively. Biomass increased steadily during the destratification process while production remained constant. Production then increased steadily during the destratification process while production remained constant. Production then increased two to three fold in twelve hours during the period of maximum water column homogeneity. Increased vertical mixing and possibly stimulation of phytoplankton production ar hypothesized as the major cause of this bacterial reponse to destratification.

Some estuarine hydrographic regimes may oscillate between the moderately stratified and vertically homogeneous condition. This process is typified by the York River and other lower Chesapeake Bay subestuaries, in which the periodic oscillations are related to the spring-neap tidal cycle (Haas, 1977, Haas et al., 1980a). This oscillatory behavior is manifested as successive stratification and destratification cycles in which each destratification even correspond to the predicted high spring tide. Apparently the increased turbulent mixing associated with peak spring tidal currents causes destratification about 1-6 days after the high tide peak (Haas, 1977). The destratified water column, characterized by top-to-bottom salinity differences of less than 1.00 °/oo, usually persists for about 4 days. This predictable physical event, which causes large scale hydrographic changes throughout the tidally influenced section of the estuary and occurs over a period of several days, is an ideal system for invetigating the relationship between physical and biological processes.

Destratification resdistributes ammonium and phosphate suplied from the benthos and oxygen supplied from the surface (Webb and D'Elia 1980). Destratification-restratification cycles and corresponding nutrient and oxygen redistribution processes provide a physical-chemical context in which to view biological events occurring in this type of dynamic estuarine system. The pulsing of nutrients into the euphotic zone during destratification, and the subsequent restabilization of the system has been hypothesized to stimulate phytoplankton blooms (Webb and D'Elia 1980). Development of a large population of the red tide dinoflagellate *Cocchlodinium heterolobatum* was observed during a destratification-restratification cycle in August, 1978 (Haas *et al*., 1980b).

The recent development of precise and easy techniques for measuring bacterial biomass (Hobbie *et al*. 1977) and production (Fuhrman and Azam 1980) make it possible to resolve bacterial dynamics on time and space scales relevant to estuarine dynamics. In this report I present the results of an investigation of bacterial biomass and production undertaken during a destratification even at the York River mouth in August-September, 1980.

Materials and Methods

All sampling was carried out from the RV RETRIEVER anchored in the mouth of the York River (at station B in Figure 1) during the period immediately preceding and following the spring high tide peak on August 28, 1980. Samples for detailed water column bacterial abundance and biomass determinations were taken from pumped samples at intervals of two meters, preserved in 2% formalin and stored at 3°C. Samples for bacterial production determinations were taken from standard Niskin bottle casts at high or low slack tides and processed immmediately at 25°C.

Bacteria were enumerated and cell volumes were estimated using acridine orange direct counts (Hobbie *et al*. 1977) with a Nikon Optiphot microscope equipped with 100-w Halogen epifluorescent illumination and a

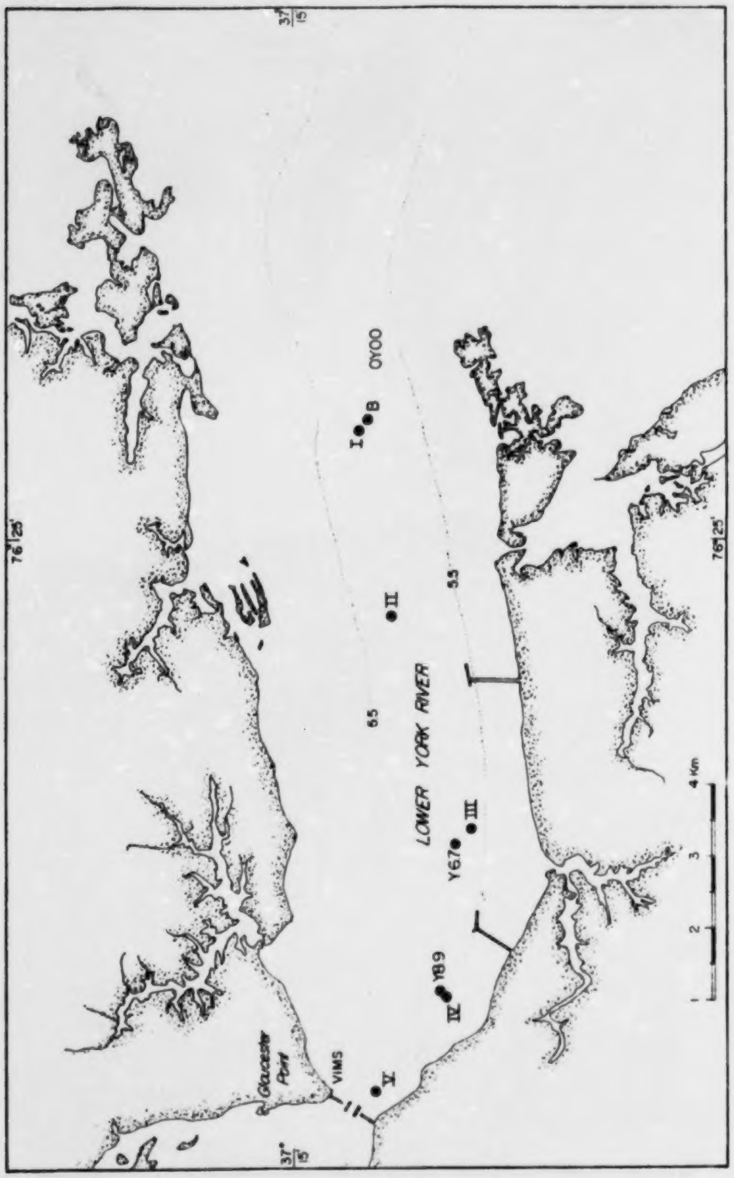

Figure 1. The York River estuary in Virginia and lower Chesapeake Bay. All work reported here was carried out at station B in the mouth of the river.

B filter unit, at a magnification of 1000 X. Free bacteria and particles were enumerated in 10 fields, and attached cells were enumerated on at least 20 particles. Only cells in direct contact with particles were judged to be attached cells.

Total bacterial biovolume was estimated in AODC preparations by examining all the bacterial cells in five microscope fields. Bacterial cells were assigned to one of four size classes: 0.4, 0.6, 0.8 µm diameter equivalent spheres, and cylinders of specified length and diameter, with reference to an ocular micrometer. Total cell volume was calcualted by multiplying the abundance in each size class by the appropriate volume, and summing the total volume in each size class. Mean cell volumes were calculated by dividing the total volume by the total count. Carbon biomass was computed from volume using the conversion factor 1.1×10^{-13}gC μm^{-3} (Watson _et al_. 1977). This procedure works relatively easily and is not subject to cell shrinkage errors introduced during critical point drying for electron miroscopy (Fuhrman _et al_. 1980). Cell volume estimates were made for each sample on which production measurements were made.

Coefficients of variation for duplicate total counts averaged 10%, and averaged 30% for volumes, due to patchy distribution of large cells.

Bacterial production rates were estimated from the rate of incorporation of ^{3}H-thymidine into cold 5% trichloroacetic acid (TCA) extracts of incubated water samples, as described by Fuhrman and Azam (1980). Duplicate of triplicate 10 ml water samples were incubated with 5nM ^{3}H-menthyl thymidine (New England Nuclear, specific activity 71.8

Ci mmol^{-1}) for 30 min. Samples were then chilled in ice, extracted in ice-cold 5% TCA, filtered onto 0.2 µm Gelman membrane filters, rinsed with 1-2 ml ice-cold 5% TCA, and placed in scintillation vials. Abiotic adsorbtion controls for each sample were prepared by poisoning with 1 µM $HgCl_2$ and were otherwise treated like the live samples. Radioactivity retained on the filters was assayed on a Beckman LSC 100 scintillation counter. Quenching was determined by the external standard ratio method. Rates of cell production were calculated by multiplying the rate of exogenous ^3H-thymidine incorporation into TCA insoluble material (moles thymidine l^{-1} d^{-1}) by 3.5×10^{17} cells mole thymidine incorporated^{-1}. This factor is based primarily on the median of published estimates of DNA per bacterial cell ($0.75 - 4.8 \times 10^{-15}$ g cell^{-1}; Fuhrman and Azam, 1980) and on the assumption that 80% of the TCA insoluble lable is DNA. Carbon production rates were calculated by multiplying the cellular production rates by mean cell carbon for each sample. Coefficients of variation for thymidine uptake averaged 25%.

Amino acid uptake was estimated using a tritiated amino acid mixture (New England Nuclear). Experiments were performed similarly to the thymidine assays, with 0.5 µCi of the mixture added in 0.1 ml to each 10 ml sample. Turnover times were estimated from the fraction of the total label added which was assimilated into the bacteria (Azam and Holm-Hansen, 1973).

Physical parameters and oxygen concentrations were measured with an STD and in situ YSI oxygen electrode. Nutrient concentrations were measured on pumped samples according to Technicon Autoanalyzer techniques by K.L. Webb and C.F. D'Elia.

Results

The spring tide peak of +3.0 ft occured on August 28 at 0200, and vertical salinity profiles showed a top-to-bottom salinity difference of less than 1.00 $^{o}/oo$ by August 29 at 2100. The destratification process culminated in a minimum Δ S $^{o}/oo$ of 0.26 - 0.33 on August 31 - September 1. Vertical mixing causing destratification occured in situ, not as a result of an advected water mass (L.W. Haas, pers. comm.). Vertical hydrographic and oxygen profiles characteristic of stratified and destratified water columns were depicted in Figure 2A and C. Ammonium and oxygen were redistributed in the water column during the destratification process (Fig. 3) although these profiles are complicated somewhat by high-low tide differences and diel patterns related to photosynthesis.

Vertical profiles of bacterial abundance and per cent attached bacteria show that bacteria were stratified in the stratified water column (Fig. 2B0 and vertically homogeneous in the destratified water column (Fig. 2D). Prior to destratification, the proportion of attached cells was extremely high (80-90% attached) in the mixed layer and declined with depth under the pycnocline (Figure 2B). In the destratified estuary, the relative abundance of attached cells was more characteristic of coastal water (10% attached cells) and was vertically homogeneous (Figure 2D). Most particles were flocculent organic

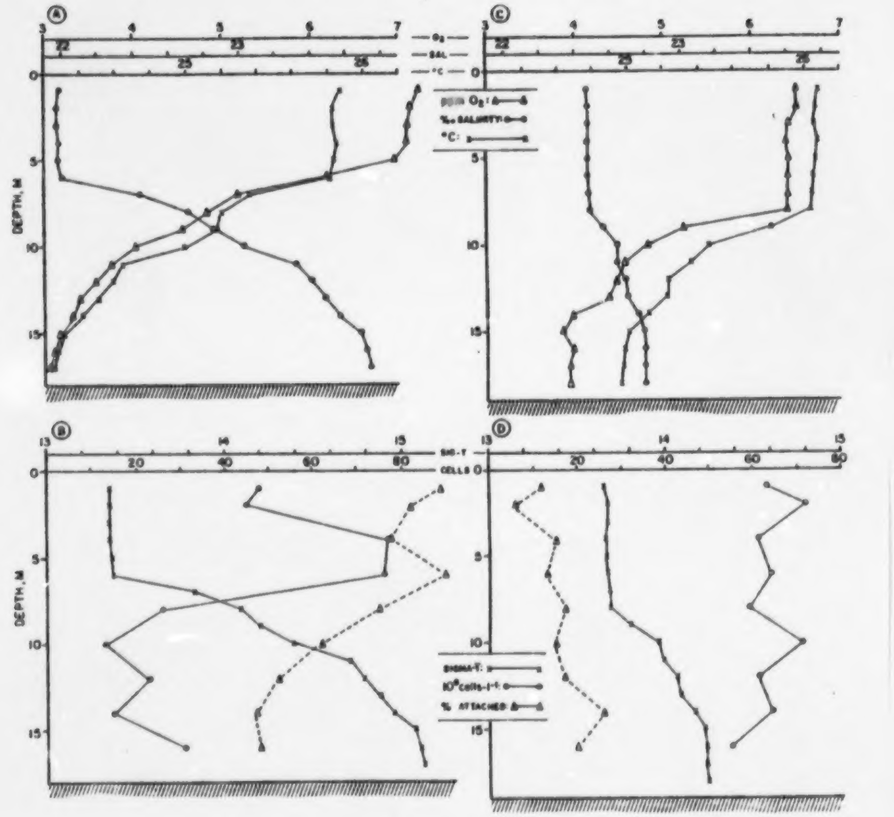

Figure 2. Vertical profiles of oxygen, salinity and temperature (A and C), and of sigma-t, total bacterial abundance and percent attached bacteria (Band D). A,B: Stratified water column on 28 August 1980, 1847 hrs. C,D: Destratified water column on 30 August 1980, 2045 hrs.

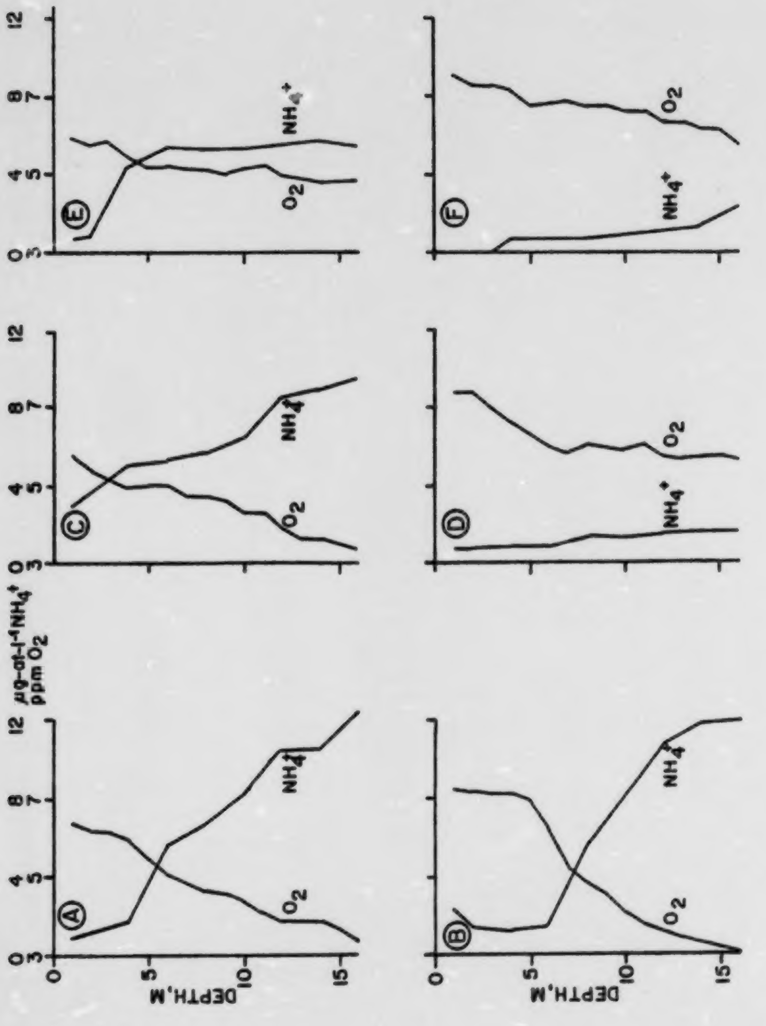

Figure 3. Vertical profiles of oxygen and ammonium. A: 28 August 1980, 0730 hrs, high slack tide (HS). B: 28 August, 1842 hrs, HS. C: 30 August, 0900 hrs, HS. D: 30 August, 1430 hrs, low slack tide (LS). E: 1 September, 1030 hrs, HS. F: 1 September, 1600 hrs, LS.

aggregates 10-40 µm wide before destratification and most were smaller clay particles after destratification. As indicated by Figure 2, integrated free and total bacterial abundance and particle abundance increased during destratification while particle colonization and integrated attached bacterial density decreased (Table 1).

Distribution of cell sizes followed the expected pattern (Ferguson and Rublee 1976), in which over 90% of the cells were 0.4 - 0.6 µm cocci and coccobacilli. The proportion of smaller cells was slightly lower at the two low slack tides measured. Cell volume was more evenly distributed in each size class at low tide, and there were pulses of larger cells at several times and depths. Generally however, little variability in the distribution of cell sizes with time or depth was observed.

Rates of thymidine incorporation into cold TCA insoluble fractions remained constant or increased slightly with depth, and increased with time and destratification (Figure 4). Cell abundance decreased or remained constant with depth, and also increased with time (Fig. 4). The specific activity of the cells (moles thymidine cell^{-1} day^{-1}) increased with depth, although not always steadily (Figure 4). Cell abundance and specific activity also increased with time, as destratification proceeded. Amino acid turnover times were lower prior to destratification and then remained relatively high in the destratified estuary. No significant correlation between amino acid and thymidine uptake was observed. The increase in amino acid turnover times may have been due to an increase in the size of the amino acid pool as a result of

TABLE 1. Attached bacteria and particle data for vertical profiles of stratified and vertically homogeneous water column.

	AUGUST 28, 1847 hr		AUGUST 30, 2045 hr	
depth	cells particle^{-1}	particles ml^{-1}	cells particle^{-1}	particles ml^{-1}
1	73	6.0	3	25.1
2	85	4.3	2	21.7
4	79	7.5	4	23.4
6	129	5.3	3	28.4
8	26	7.4	3	33.4
10	30	2.3	3	36.8
12	8	15.0	3	35.1
14	2	35.1	4	41.8
16	7	21.7	3	36.8

Integrted Abundances	August 28	August 30
10^{12} free cells m	6.9	46.2
10^{12} attached cells m	26.1	9.3
10^{12} total cells m	33.0	55.5
10^{12} particles m	10.5	28.3

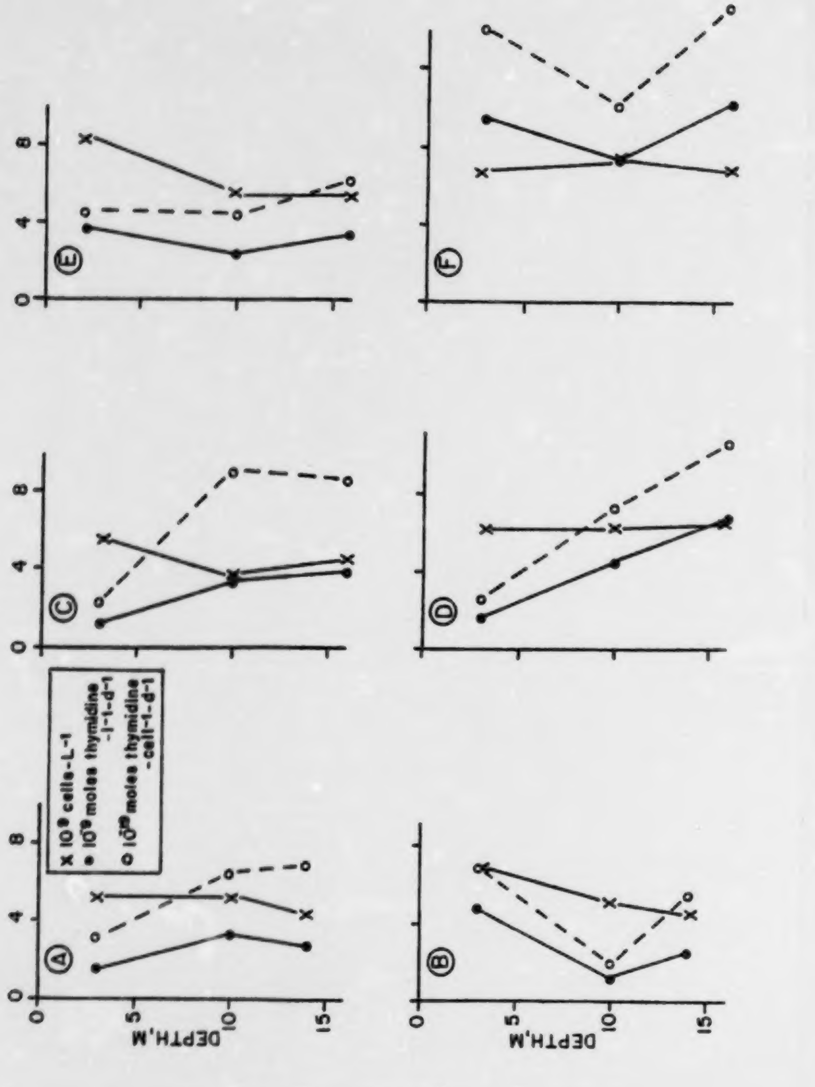

Figure 4. Vertical profiles of cellular abundance, thymidine uptake into cold TCA insoluble material, and specific activity of thymidine uptake. Dates and times as in Figure 3.

amino acid flux from the benthos.

Discussion

The picture of bacterial distributions and dynamics presented here differs in several striking ways from those for other systems. Most obviously, the York River system appears to shift from domination by very high proportions of attached bacteria (40-90% on August 28, Fig. 2) to high proportions of free bacteria (>80% on August 30). The decrease of the attached population during destratification is probably related more closely to particle quality than to surface area since the total particle abundance increased nearly threefold in the same time period (Table 1).

Prior to destratification, vertical bacterial distribution appeared to be influenced by hydrographic structure, with high total numbers and high % attached values found in the surface mixed layer and decreasing numbers below the pycnocline (Figure 2B) and thermocline (Figures 2A and 2B). Ferguson and Palumbo (1979) observed that total bacterial numbers increased sharply at the thermocline in stratified waters of the New York Bight. These contrasting distributions may not have entirely unrelated explanations. Ferguson and Palumbo (1979) suggested that suspended particle become concentrated as they sink through the pycnocline, leading to increasing cell abundance. In the stratified York River, the inflowing tidal water under the thermocline is characterized by more small particle with fewer bacteria per particle. The outflowing river water in the surface layer however, is transporting large organic detrital particles with many bacteria per particle. These flocculent particles are held in suspension by the strong physical gradients in the stratified water column (Figure 2A). The high numbers of cells in the fresher surface water prior to destratification suggest that the river upstream of the estuary is an important source of bacteria, which may be somewhat less active than the indigenous estuarine forms in the bottom water (Figure 4).

The response of the bacterial community to destratification is summaried by integrating water column biomass and production rates during the event, as shown in Figure 5. Decreasing stratification is indicated in the sigma-t vs depth plots in Figure 5A. Cellular abundance (Figure 5B) and bacterial carbon biomass (Figure 5C) increase steadily throughout destratification, at approximate rates of 13 x 10^{12} cells m^{-2} d^{-1} and 0.13 gC m^{-2} d^{-1}, respectively (computed from linear regressions with r^2 = 0.93 and 0.82 respectively). Meanwhile, thymidine based production rates remained constant at 30-40 x 10^{12} cells m^{-2} d^{-1} and 0.2 - 0.4 gC m^{-2} d^{-1}; respectively, until September 1, when production rose substantially. This increase in cell production coincided with the maximum homogeneity of the water column. Thymidine based rates were all higher than the observed rates measured from increases in cell numbers and bacterial carbon in this open system. This suggests that the thymidine method does yield rates high enough to account for the observed growth in the system. Furthermore the data suggest that some newly produced bacteria may continually be lost from the system, probably via sinking, export, or predation.

Specific growth rates for the bacterial assemblage averaged 0.4 day^{-1} for the period 28-30 August when an increase to 1.1 day^{-1} was observed. These rates suggest that with the measurements I employed, no biological response to tidal mixing could be seen in less than 2-3 days. The increase in growth rate was observed 3-5 days after the maximum tide, during the period of greatest vertical homogeneity. Apparently, the bacterial population can respond to such mixing events and is capable of doing so on the time scale demanded by the physical oscillation period of the system. A similar rapid, substantial increase in bacterial activity has been observed following a tropical storm in the Gulf of Mexico (R.L. Ferguson, pers. comm.).

The reason for this response of bacterial populations to destratification and vertical mixing is not clear. Several aspects of the data presented here are consistent with the hypothesis that the York River estuary is a system dominated by benthic-water column exchange processes. River bed oxygen consumption and ammonia flux are enhanced during destratification, and ammonium regeneration strongly influences ammonia concentration in the water column (Phoel et al. 1980). Vertical profiles of oxygen and ammonia (Figure 3), and thymidine uptake (Figure 4), all indicate greater biological activity in the lower water column. Destratification produces a vertically homogeneous water column and is accompanied by increased vertical exchange (Haas 1977; Haas et al. 1980a) and nutrient and oxygen redistribution (Webb and D'Elia 1980; Figure 3). During the destratification event, particle density increased threefold (Table 2), suggesting mixing off the bottom. Mean biomass per cell was

Table 2. Turnover times of dissolved amino acids due to heterotrophic assimilation of a ^3H-amino acid mixture by bacteria.

date/time/depth	T_t, hours
8-28/0730/3	1.0
/10	1.0
/14	1.4
8-28/1847/3	1.5
/7	7.1
/14	0.9
8-30/0900/3	3.6
/10	12.5
/16	11.1
8-30/1430/3	7.7
/10	20.0
/16	4.8
9-01/1030/3	20.0
	10.0
	-
9-01/1600/3	3.1
/10	4.3
/16	2.9

generally uniform at 9 + 2 fg $cell^{-1}$ throughout destratification, except for a pulse to 15 fg $cell^{-1}$ on August 28 at 1847 hrs (Figure 5D). This pulse of large cells may have been due to mixing of cells off the bottom following the spring tide peak (8/28 at 0200), although cell size shows no consistent distribution with depth or tide state. Mineral silica increased significantly in the water column following the tide peak, then declined, also suggesting vertical mixing (D.M. Nelson, pers. comm). Yet how this mixing might stimulate bacterial production and growth is not evident from this data. Neither oxygen nor ammonia were in extremely short supply prior to destratification, so the redistribution of those species seems unlikely to have stimulated bacterial growth directly. However, in a subsequent vertical mixing event in upper Chesapeake Bay, oxygen redistribution greatly enhanced ammonium oxidation to nitrite, a highly aerobic process (J.J. McCarthy, pers. comm). Vertical mixing and nutrient redistribution may have stimulated phytoplankton growth, which in turn stimulated bacterial growth. Phytoplankton responses to destratification have been observed in this system (Haas et al. 1980b). Bacterial uptake of excreted photosynthetically fixed labelled carbon and bacterial growth and abundance have been observed to track phytoplankton blooms (Hagstrom et al. 1979). Resuspension of diatoms from the benthos and subsequent stability trigger diatom blooms in continental shelf waters (Walsh et al. 1978; Malone and Chervin 1979). During this destratification event biogenic silica (diatoms) increased dramatically in the water column following destratification and maintained high levels

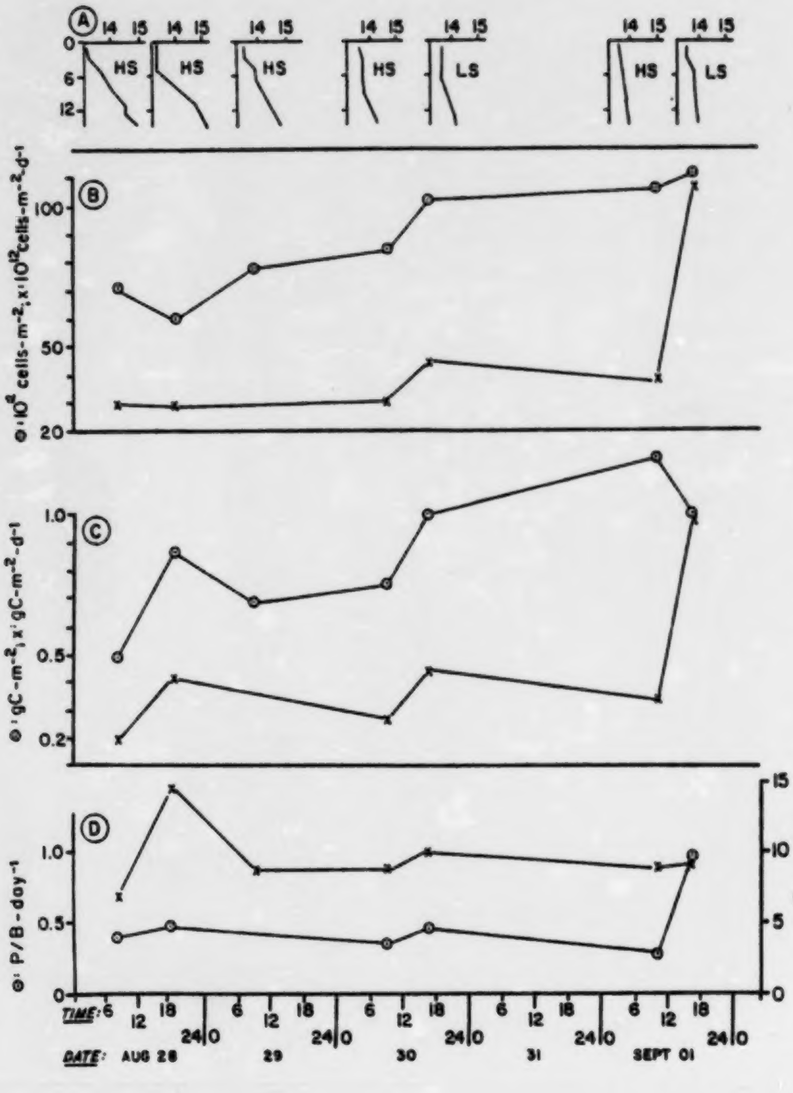

Figure 5. Integrated daily water column bacterial cell abundance, biomass, and bacterial production. A: sigma-t profiles for each sampling time. B: integrated cell abundance (o-o) and production (x-x). C: integrated bacterial carbon biomass (o-o) and production (x-x). D: bacterial turnover rates (o-o) and mean cell masses (x-x).

throughout our observations (D.M. Nelson, pers. comm.). However no other evidence of phytoplankton activity for this period is available. Further documentation and elucidation of biological responses to vertical mixing and destratification in this and other systems may be most profitably accomplished by utilizing predictable physical events such as the spring tidal mixing event documented here.

Physical and biological interactions of this type may have significant repercussions for water quality, by controlling nutrient, carbon, chlorophyll and organic matter export from estuaries into the ocean. These events may also influence the dynamics of river plumes, river-ocean exchange processes, and productivity patterns across the continental shelf.

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CHAPTER IV

Convenient, Reliable Chlorophyll Extraction from Marine Phytoplankton with a Dimethyl Sulfoxide:Acetone:Water System

D. Hayward

K.L. Webb

ABSTRACT

Various filters, handling methods, and three solvent systems were compared in the laboratory for quantity of chlorophyll a type fluorescence activity extracted from natural phytoplankton populations and cultures of chroococcoid cyanobacteria, a diatom, and a chlorophyte, all marine or estuarine species. The results suggested that Whatman GF/F glass fiber filters and extractions without grinding, at ambient temperatures, in a solvent system composed of dimethyl sulfoxide:acetone:water 45:45:10(v/v/v) produce equal or greater fluorescense activity than other combinations tested. A field study was conducted comparing the results from this method with those from a ''traditional'' method involving freezing filters, grinding and refrigerated extraction in 90% acetone. Results from this study indicate that means values produced by the two methods are not significantly different, while the precision of the proposed method is somewhat better, and storage of material extracted by this method over extended periods produces no significant change.

Recently a variety of solvent systems containing dimethyl sulfoxide (DMSO) has been suggested for the extraction of chlorophyll type pigments from phytoplankton. Reports have indicated that a solvent system composed of DMSO and 90% aquaeous acetone in a 1:1 ratio has extractive characteristics superior or equal to 90% acetone, 100% methanol, and methanol:N,N-dimethyl foramide (1:1) systems for some types of freshwater phytoplankton (Shoaf and Lium 1976; Stauffer et al. 1979). Shoaf and Lium (1976) have reported the virtual identify of absorbance spectra of chlorophylls a and b in 90% acetone and DMSO:acetone systems and demonstrated the validity of using standard equations for chlorophyll estimation with DMSO:acetone extracts. Recently, Burnison (1980) has described a method using pure DMSO at 65° followed by dilution with 90% acetone.

There also has been wide variation in filters and handling methods used for chlorophyll a biomass estimtes. Filters used have included membrane filters (Shoaf and Lium 1976; Strickland and Parsons 19722) and glass fiber filters (Holm-Hansen and Riemann 1978; Stauffer et al. 1978) of varying nominal pore size. Sheldon and Sutcliffe (1969) reported incomplete retention of particles in the 0.5-1 um size range for many of these filters. However, significant numbers of chroococcoid cyanobacteria of this size have been reported by Waterbury et al. (1978) and Johnson and Sieburth (1979), consequently suggesting the possibility of biomass understimation. Extraction techniques have included grinding (Shoaf and Lium 1976) and not grinding (Stauffer et al. 1979; Holm-Hansen and Riemann 1978), freezing (Holm-Hansen and Riemann 1978), extraction periods from 10 min (Burnison 1980) to 36 hr (Stauffer et al. 1979), and refrigerated (Stauffer et al. 1979), ambient temperature (Shoaf and Lium 1976), and heated (Burnison 1980) extractions.

No work has been published concerning the use of the DMSO:acetone system with marine plankton species, although Seely et al. (1972) reported using DMSO as part of a serial extraction method for brown algae. Here we report the results of a series of extraction experiments which compared handling methods, solvent systems, and filters in order to select a less complex, more convenient, but still reliable procedure suitable for marine and estuarine phytoplankton.

Filters tested were (1) Whatman GF/F, (2) Whatman GF/C, (3) Reeve Angel 934AH, (4) Nuclepore polycarbonate 0.2 um, (5) Gelman type A/E, (6) Millipore GSWP025, .22 um. Reagents used were (1) Acetone, Malinkrodt, SpectrAR grade (2) Methyl Sulfoxide (DMSO), Malinkrodt, analytical reagent grade, (4) Diethylamine, Fisher Scientific Company, reagent grade, lot 766108. Fluorescence measurements were taken with G.K. Turner Associates model 111's fitted with a standard or a ''high sensitivity'' door, F.4T4-blue or F.4T4-clear quartz lamp, a Corning CS.5-60 filter for excitation light and a Corning CS.2-64 filter for emitted light.

Phytoplankton for the initial laboratory study were (1) undefined populations from the York River (USA) taken in May and June 1980, (2) a mixture of two cultures of chroococoid cyanobacteria (*Synechococcus sp*. $1.6x10^6$-10^4 cells ml^{-1}, 0.5x1.0 um in dimension), and cultures of (3) *Chlorella sp*. ($194x10^4$ cells ml^{-1}), a chlorophyte, and (4) *Thallasiosira sp*. ($10.75x10^4$ cells ml^{-1}), a diatom. Samples for the field study were taken in the York River (USA) and Lower Chesapeake Bay, July 8-16, 1980.

The solvent systems used were (1) DMSO:acetone:water 9:9:2 (DA10), (2) DMSO:acetone:water 10:9:1 (DA5), (3) acetone:water 9:1 (A10). ALL proportions are by volume. DEA at 0.1% by volume was routinely added to DA10 and DA5 (Staufer et al. 1979).

The following is the standard extraction procedure now used. A measured volume of sample sufficient to produce visible color on the disc is filtered through a Whatman GF/F 2.5 cm filter. For estuarine water 10 ml is usually sufficient. The filter is then folded and placed in a 16x100 mm glass culture tube which is coated (see below) to exclude as much light as possible. The tube contains a premeasured 10 ml aliquot of DA10. The tube is closed with a teflon lined screw cap and the filter is extracted for at least 2 hours at ambient temperature. Filters are always manipulated with forcceps. It has not been necessary to filter or centrifuge the sample before mesuring fluorescence. Extracting material has been stored at ambient laboratory tmeperatures for up to 32 days with no sifnificant change in the results.

Several variations of this method were tested. Refrigerated extractions were carried out at 4°C. A10 extractions were refrigerated and were allowed to continue over 24 hr. Ground extractions were carried out as follows: The filter was folded and placed in a glass tissue homogenizer and covered with solvent. The filter was then ground with a teflon pestle until it was completely disintegrated. The pestle was rinsed with solvent and the slurry was poured into a 15 ml graduated conical centrifuge tube. The homogenizer was rinsed and the rinse material was added to the tube. The volume in the tube was brought to 10 ml and the tube capped with paraffin film. After the extraction period was complete the tubes were centrifuged in preparation for decanting. All A10 extractions were ground. When filters were frozen, they were folded, individually wrapped in aluminum foil, and placed in a freezer at -20°C for from 2 hours to 30 days.

After the extraction period, the solvents were decanted into cuvettes and fluorescence was measured. The coefficients of fluorescence for chlorophyll a in the solvents were determined from a chlorophyll a standard, Sigma Chemical Company, product no. C-5753, lot number 39C-9690. The Sigma standard was dissolved in 100% acetone and then diluted so that final concentrations of solvents matched those of the extraction systems.

The pigment concentration values used to calculate the comparisons between methods were chlorophyll a equivalents, with no correction. Because of probable multiple interferences from chlorophylls b and c, no correction was judged appropriate (Gibbs 1979). Because sample variance was significantly correlated with sample mean (Table I), a log transform was performed before analysis (Snedecor and Cochran 1967 p 329). All analyses were performed using Statistical Analysis System GLM, CORR, SUMMARY, and MEANS procedures (SAS 1979).

Table I. Correlation analysis of sample mean and variance. Initial comparisons consist of sample of cultures and York River sample of May and June, 1980; field study samples are from lower Chesapeake Bay and the York River during July 1980.

Samples	R	PROB> R under HO:RHO=0	N
Initial comparisons	0.409	0.0277	29
Log_n Initial comparisons	0.079	0.6826	29
Field Study	0.569	0.0001	136
Log_n field study	0.008	0.9277	136
Overall	0.422	0.0001	165
Log_n overall	-0.068	0.3855	165

As a means of excluding light from the culture tubes during extraction, the tubes were dipped twice in a mixture of lampblack and plastic ''tool grip compound'' obtained from Brookstone Company, Peterborough, NH. About 70 cc of lampblack was added to each 16 oz. can of red compound and mixed thorougnty. Approximately three dozen tubes were coated from each can.

Before plankton extractions were initiated, 3 untreated filters of each type were extracted for over 72 hr in 5 ml of each solvent to determine background fluorescence levels. The results showed variable fluorescence activity derived from the Millipore filters of 07-2.9 ugl-1 uncorrected chlorophyll a equivalents for a 5 ml sample in all solvents. Therefore these filters were not used in the subsequent tests. All others produced activity below 0.11 ugl-1.

Initially, comparisons were made in the laboratory between solvents, filters, and handling methods (treatments). A total of 116 observations were made, and the results subjected to analysis of variance. The variation due to all factors was significant (Table II); the combination of Whatman GF/F filters, DA10 solvent, and ambient temperature extraction without grinding consistently produced values equal to or greater than other techniques.

Table II. Initial comparisons. Analy sis of variance of $\log_n$ of uncorrected chlorophyll a equivalents. Samples are from cultures and from the York River during May and June, 1980. n = 116, cells = 2-7.

Source	df	SS	F	Pr>F
Population	8	161.89	4379.4	0.0001
Solvent	1	0.6	12.8	0.0005
Filter	4	0.11	5.7	0.0004
Treatment	3	0.49	3.5	0.0176

The above results encouraged us to undertake a more extensive comparison between our recommended method and the traditional method of grinding with A10 after the filter had been frozen and stored. The samples were taken July 8-16, 1980. Repetitions intended for A10 were taken on Whatman GF/F filters, frozen, and ground and extracted in the laboratory. DA10 repetitions were also taken on Whatman GF/F filters, but were extracted immediately in 5 ml of solvent.

A total of 272 observations were made. The results (Table III) indicate the variance due to method is not significant. A t-test of the differences between means was not significant (P=0.94). As a comparison of the precision of the methods, the means of variances were subjected to an F-test (Snedecor and Cochran 1967 p116). The precision of DA10 extractions was significantly different (P-<0.05) from and better than the A10.

Table III. Statistical results from the field study. Data consist of log transformed uncorrected chlorophyll a equivalents, n = 272, cells = 2. n

Analysis of Variance

Source	df	SS	F	Pr>F
Method	1	4.52×10^{-5}	0.01	0.9225
Sample	67	4.47×10^{1}	13.97	0.0001

Analysis of Precision

	MEAN	MEAN VARIANCE	F
Log of all observations	1.8272	—	
Log of A10 extractions	1.8285	0.0315	
Log of DA10 extractions	1.8259	0.0182	1.734*

*P=<0.05

To test the effect of storage on extracting material, the second repetition of some of the DA10 extractions were stored in the original sample tubes at room temperature for varying periods up to 32 days after the first repetition was read. The results (Figure 1) indicate no significant change in the determined values ($P=0.99$), nor in coefficient of variation associated with the interval of storage ($P-0.55$).

Extractions are very rapid in DA. Depending on the population, extraction in DA was 90-100% complete after 15 min. The diatoms were completely extracted after 5 min., the chlorophytes after 15. A mixed population from the York River was more than 95% extracted after 15 min. Complete extraction of pigment from the chroococcoid cyanobacteria varied with the concentration of organisms, possibly due to packing in the filter, from 5 to 30 min. Very low concentrations, around 1 ugl^{-1} of pigment in a 5 ml sample, were 95-100% complete after 5 min. Heavy concentrations, >80 ugl^{-1}, required 30 min. In all cases, no significant further extraction was noted after 2 hr, although no degradation was noted if extraction was allowed to continue for extended periods.

It was noted that extractions with DA5 required an unusual amount of acid to achieve a stable acidification ratio compared to A10, with as much as 3 drops of concentrated HCl needed. This was deemed excessive. By analogy with earlier work of Marker (1972) and Gibbs (1979) with aqueous acetone systems, it was hypothesized that a DA system which was 10% water would exhibit stable ratios with less acid. Stable ratios were achieved in DA10 with 3 drops of 2N HCl in 10 ml of solvent. Spectral characteristics of the two systems were indistinguishable and the difference in pigment recovered was insignificant, (P=0.52).

We recommend the standard method described here for extraction of pigment from marine and estuarine phytoplankton populations. The consistently greater or equal quantities of pigment produced and the convenience of the technique, with no grinding or refrigeration necessary, ease of long term storage, increased precision and the relatively rapid rate of extraction particularly support it. The method is extremely advantageous for field use as the only special handling necessary is the exclusion of light from the sample.

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CHAPTER V

Ammonium Recycling in Upper Chesapeake Bay, Spring 1980

J.F. Heinbokel

Introduction

Recent findings suggest that for much of the year inorganic nutrients limit both biomass and productivity of the phytoplankton of the Chesapeake Bay (McCarthy *et al.*, 1975) as, indeed, they apparently do in most marine systems (e.g. Parsons and Takahashi, 1973). While total nutrient loads define maximum biomass levels in a relatively straightforward manner, the relationship between nutrients and primary productivity is less clear. In the Chesapeake Bay, for instance, maximum rates of primary productivity are observed in the warm summer months when dissolved nutrients (NO_3, PO_4, NH_4) are at their lowest concentrations and often undetectable (McCarthy *et al.*, 1975).

If the high levels of productivity observed cannot be supported and maintained by the ambient stocks of inoganic nutrients, then a mechanisms must be invoked involving rapid cycling of these nutrients such that nutrients are taken up and utilized as rapdily as they are made available; flux (and therefore productivity) remains high despite extremely low levels of nutrients. Two mechanisms of nutrient supply can be envisioned in the summer when tributary input is low. The first of these mechanisms is mixing of nutrients from the sediments and deeper waters. The very strong pycnocline which develops each summer at 10-12 meters in the main axis of the Bay efficiently retards upward mixing of deeper waters; nutrient input from peripheral sediments at less than 10 meters depth, however, is a largely unquantified process. The second mechanism is the rapid *in situ* recycling of nutrients by the zooplankton. This scernario requires a very close coupling between rates of phytoplanktonic growth, grazing induced mortality, and subsequent release of nutrients directly by the zooplankton and by bacterial activity on particulate organic material. Storms (1974) demonstrated that copepod ingestion over the year was equal to an average of less than 20% of the measured planktonic primary production and that during the summer months this impact shrank to less than 10%. For the grazing hypothesis to be validated it is necessary, therefore, to identify another group of grazers capable of providing a more significant quantity of recycled nutrients; the microzooplankton are a logical group for this purpose.

Microzooplankton have been conspicuous in a number of speculative discussions of planktonic nutrient cycling: Johannes (1964) pointed out their potential importance in phosphorous dynamics; Johansen (1976) extrapolated from laboratory-derived ammonium and urea excretion rates to calculate that the ciliates at a coastal station off Nova Scotia were generally excreting sufficient nitrogen to account for measured rates of primary productivity; and Harrison (1980) suggested that the microzooplantkon should provide an increasing reaction of the nitrogen required for primary production as one moved offshore. In the Chesapeake Bay ciliates and rotifers comprise a significant microzooplanktonic assemblage throughout the year (Heinbokel, in prep.). Additionally, colorless flagellates are present in the waters (Haas and Webb, 1979), although their abundance and distribution patterns are even less well-known than those of the larger members of the microzooplankton.

Measuring rates of excretion in these microzooplanktonic grazers which cannot be conveniently separated from their food organisms has proven an especially difficult problem. In the natural conditions of greatest interest, the phytoplankton present in the samples can be expected to be limited by at least one of the nutrients of interest so that excretion by the grazer(s) may be significantly confounded by simultaneous uptake of that nutrient by the phytoplankton. Even if it were possible to conveniently isolate the protozoan grazers from their food as has often been done for the larger metazoan forms, excretion rates can be expected to change markedly due to starvation effects over the several hour period required to observe measureable changes in dissolved nutrient levels. Recent development of an extremely senstive ''isotope dilution'' technique for studying nutrient excretion, however, provides a potentially powerful tool for investigating this process in reasonably natural assemblages of protozoa and their food.

During 1980, two experiments were performed in Chesapeake Bay to examine the question of nutrient cycling. The two basic questions asked by these experiments were: 1) at what rates and with what significance was ammonium being recycled _in situ_ in surface waters? and 2) what deductions could be made as to the size of organism responsible for any measurable ammonium excretion? For these purposes a very senstive isotope (^{14}N) dilution technique (Harrison, 1978; Caperon et al, 1979) was utilized to examine both uptake and release of ammonium. During the second (July) experiment, ambient ammonium concentration were almost undetectable, possibly compromising the ''tracer'' nature of the technique; the first (May) experiment is, therefore, emphasized below.

Material and Methods

Isotope Dilution Methodology: Isotope dilution as a technique to study aquatic nitrogen dynamics was introduced by Alexander (1970) in Arctic Ponds. Harrison (1978) and Caperon et al. (1979) have described and utilized the technique in marine planktonic environments. Essentially the technique is simple and straightforward: ^{15}N-labeled ammonium is added to a natural water sample and the concentration and specific activity of the dissolved ammonium is followed over a time course. Regeneration rates of ammonium into this soluble pool (observed as a dilution of the ^{15}N fraction by newly excreted ^{14}N-ammonium) can then be calculated after making three assumptions: 1) there is no isotopic discrimination, 2) the rates of ammonium uptake and excretion must remain constant during the course of the incubation, and 3) no assimilated ^{15}N may be excreted back into the disolved ammonium pool (Caperon et al., 1979).

8 May 1980 Experiment: The protocols of experimental design are presented in Fig. 1. Surface water from the Chesapeake Bay near the mouth of the Severn River was collected with a clean bucket and placed in a large carboy. Following removal of a sample for analysis of ammonium concentration, the water was divided into two carboys, being poured through a 35 µm nylon mesh into one carboy (<35) and through no exclusion filter into the second carboy (total). Samples were removed from each of

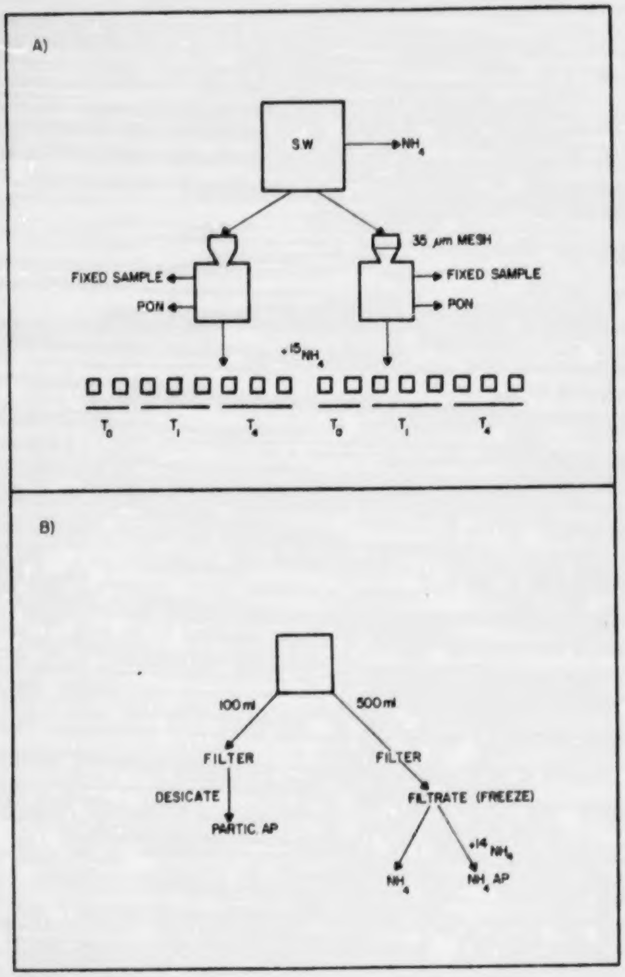

Figure 1. Experimental protocols for May 1980 experiment. A. Set-up of experimental incubations. B. Sample manipulation and analysis following incubations. SW = sea water; PON = particulate organic nitrogen. T_0, T_1, T_4 = 1-liter bottles to be incubated for 0, 1, and 4 hours; AP = atom percent ^{15}N.

these carboys for fixation with Lugol's I_2/KI fixative and for analysis of particulate organic nitrogen (100 ml onto a Whatman GF/F filter). 800 ml aliquots were then dispensed from each carboy into each of 8 1-liter Pyrex, glass stoppered bottles. 0.15 µm at NH_4-^{15}N (as ammonium sulfate, 97% ^{15}N - Koch Isotopes) was then added to each bottle. Two bottles from each set were filtered immediately (T_o); the remaining 6 bottles were placed in an on-deck plexiglass water bath through which surface water was pumped. Three bottles were incubated for 1 h (T_1) and three for 4 h (T_4) prior to filtration. All incubations were stopped by filtration. 2 100 ml aliquots were filtered onto 2.4 cm GF/F filters; filters were stored in a vacuum desicator until analysis. A 500 ml aliquot was passed through a 4.7 cm GF/F filter and the filtrate collected, frozen, and returned to the laboratory for analysis.

In the laboratory the frozen filtrate was thawed. Two 10 ml aliquots were analysed for ammonium concentration. 400 ml were placed in a 1-liter round bottom boiling flask and 2 µg at NH_4-^{14}N and 5 ml of a well-washed suspension of MgO as a buffer were added. Approximately half the sample volume was distilled over into an acid trap containing 20 ml of 0.1 N H_2SO_4. This material, containing most of the NH_4 from the original sample, was gently boiled down to a volume of 5-10 ml and placed in a 20 ml glass scintillation vial.

^{15}N analyses were performed on both the particulate and dissolved ammonium fractions with a JASCO emission spectrometer in the laboratory of W.G. Harrison, Bedford Institute of Oceanography using methods (unpubl.) developed and tested in that laboratory.

11 July 1980 Experiment: A second experiment was conducted near the mouth of the Chesapeake Bay. The experimental protocols were similar except a 20 µm exclusion mesh was used, 1.0 liter was incubated for each replicate, and incubations were carried out for 2 and 4 hours.

Data Analysis: Ammonium regeneration rates can be calculated readily from times series data on concentration of ammonium and the atom percent of ^{15}N ($AP = \frac{^{15}N}{^{15}N + {}^{14}N} \times 100$) present in that ammonium:

$$\text{Regeneration:} \; \frac{\ln\left(\frac{AP_o}{AP_f}\right)(N_o - N_f)}{\ln\left(\frac{N_o}{N_f}\right) T}$$

where AP_o and AP_f are the ^{15}N atom percentages at the start and end, respectively, of a time interval, N_o and N_f are the initial and final NH_4 -N concentrations, and T is the duration (in hours) of the incubation (Caperon et al., 1979).

Results and Discussion

Despite the numerous manipulations necessary in processing the samples from time of initial water capture to reading the ^{15}N content of the dissolved fractions, the analytical precision of the ^{15}N content of the dissolved fractions was very high (low variability within a subsamples of the same experimental bottle) as was the very gratifying agreement between the two or three replicate bottles of each treatment

(Fig. 2a).

These determinations of ^{15}N atom percentages as read by the emission spectrometer required correcting to account for the 2 µg at NH_4-^{14}N which was added to bring the total N content of the sample up to a level which could be read by the spectrometer. Conversion of the raw ^{15}N atom percents (Fig. 2a) to the actual values characteristic of the bottles during the experimental incubations required knowledge of the original NH_4^+ concentrations in each bottle (Fig. 2b). Precision of NH_4^+ determinations at concentrations of less than 0.5 µg at NH_4-N/ℓ is considerably less than that for the original ^{15}N determinations. This lesser precision is reflected in the calculated values of the orginal ^{15}N atom percentages presented in Fig. 2c.

By combining the time course information on ^{15}N content and the ambient ammonium concentration (requiring a second use of the relatively low precision data on ammonium concentration) by means of the formula presented above it is possible to calculate the regeneration rate of ammonium in these bottles. The results are presented in Table 1.

Several points deserve discussion. First, the initial regeneration rates, 0.127-0.129 µg at NH_4-N/ℓ/h, are quite significant, implying turnover times for the dissolved ammonium pool on the order of three hours. Subsequent calculated rates are considerably lower, although this does not necessarily mean that the true rates of regeneration have been reduced. Recall that the third assumption in this analytical treatment requires that none of the added NH_4-^{15}N be taken up and then recycled back into the dissolved ammonium pool. With an initial ammonium turnover

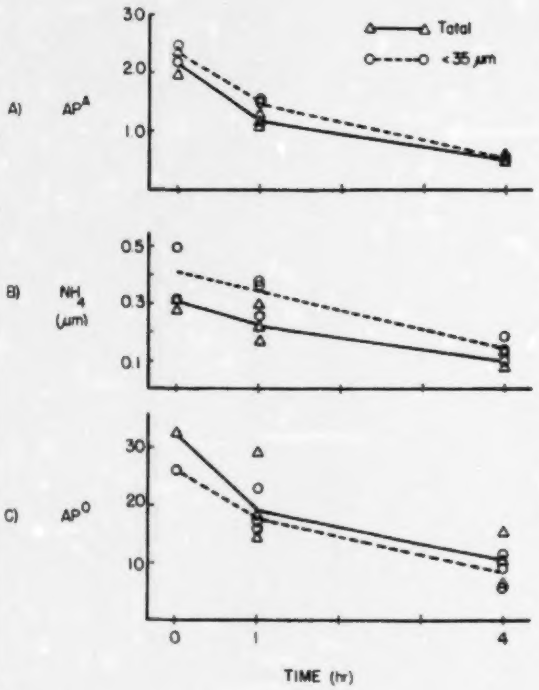

Figure 2. Time course of ammonium regeneration. A. Atom percent ^{15}N in the ammonium samples read by emission spectrometry (AP^{A}). Lines through means of replicate values. B. Ammonium concentration during experiment. C. Atom percent ^{15}N in the ammonium of the experimental bottles (AP^{O}). These values derive from those plotted in A but have been corrected for the 2 microgram atom ^{14}N - NH$_4$ per liter added to the sample prior to the ^{15}N analysis.

TABLE 1: Ammonium Regeneration Rates (μg at NH_4-N/ /h), May 1980.

	Plankton Fraction	
Time	**< 35 μm**	**Total**
0-1 hr	0.127	0.129
1-4 hr	0.054	0.034
0-4 hr	0.067	0.054

time of approximately three hours, it is likely that this assumption was violated during the full four hour incubation. Such a violation would act to reduce the calculated regeneration rates from the true rates. This may, indeed, be a problem even during the initial hour of the incubation; any such problem in the initial period would, of course, act to make these initial calculated rates conservative or minimum estimates.

The second point of discussion stems from the observation that the calculated regeneration rates are virtually identical regardless of whether the water was prescreened through a 35 µm mesh. This suggests that virtually all the ammonium regeneration in these samples is due to the action of organisms able to pass such a mesh, e.g. the bacteria, heterotrophic flagellates, ciliates, and the smallet of the metazoa such as rotifiers and perhaps naupliar copepods.

Finally, note that ammonium regeneration in the incubation bottles is not in balance with ammonium uptake; the dissolved ammonium concentration decreases throughout the course of the incubation. Several possible explanations can be advanced, although at this point these must be conjectural and are intended mostly to point toward some potentially rewarding avenues of future research. First, this imbalance may be due to experimental artifact. Venrick et al. (1977) showed that ciliates disappeared rapidly from 250 ml bottles during 12-24 h primary productivity incubations; a similar mortality in these shorter incubations would decrease the amount of NH_4 excreted into the water. Second, it is possible that a source of NH_4 regeneration has been excluded from these experiments. Inputs of NH_4 from beaneath the pycnocline, from peripheral sediments, and from larger organisms (e.g. gelatinous zooplankton and fish) have all been excluded from this experiment by means of incubating small water parcels in bottles. Third, if the planktonic primary productivity is ammonium limited, it is possible that the added ammonium acted as an enrichment rather than as a tracer and that ammonium uptake, therefore, was elevated above the rate which would characterize the natural environment.

Finally, the incubated samples may, in fact, accurately reflect the ammonium dynamics of the Bay. Ammonium concentrations may not be in a steady-state condition, or alternaticly, they may be in a steady-state but with uptake and regeneration balancing on a different time scale than the 1-4 hour incubations performed here. Mopper and Lindroth (1981), for example, found that ammonium concentrations in a set of Baltic samples increased during the afternoon and evening and decreased during the morning. Any scenario in which phytoplankton uptake increases during the day and/or regeneration processes (e.g. zooplankton grazing and excretion) increase during the night can result in qualitatively similar data.

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CHAPTER VI

Sources and Sinks of Nitrite[1].

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John L. Nevins

Abstract

The transformations of inorganic nitrogenous nutrients that are responsible for the frequently observed high levels of nitrite in the Chesapeake Bay and York River were investigated using a combination of ^{15}N tracer techniques and assays of chemical properties (NH_4^+, NO_2^-, NO_3^-, O_2, N_2O and CH_4).

During a destratification event in the York River, uptake and remineralization of NH_4^+ followed a diel cycle, but nitrification was not as closely coupled to the light regime. In addition, the observed distributions of N_2O, NO_2^-, and CH_4 in the York River suggest that the source of N_2O and NO_2^-, both produced during nitrification, was not in the river sediments. Rates of nitrification inferred from N_2O gas flux calculations are consistent with measured in situ rates.

Experiments conducted at a series of stations in the mid-Chesapeake Bay were designed to look at N-transformations among N pools and to measure the yield of N_2O-N per unit NO_2^--N during nitrification. Yield values ranged from 0.2% - 0.7%, in agreement with laboratory results (Goreau et al. 1980).

Our ^{15}N data indicate that oxidized N can be formed within the water column of the Bay, when physical events cause the mixing of NH_4^+-rich bottom water with more oxygenated surface layers. Surface waters showed unexpectedly rapid rates of NO_3^- reduction to NH_4^+.

The magnitude and duration of high concentrations of N_2O and NO_2^- in these estuarine waters during mixing events might be expected to increase if anthropogenic loading of nutrients causes anoxic conditions in the Bay to become more widespread.

Introduction

Predictable seasonal patterns of nitrogenous nutrient distribution in the northern portion of the Chesapeake Bay and the Potomac River were documented through several years of study by Carpenter et al. (1969). These and more recent data reported by McCarthy et al. (1975, 1977) suggest that for the main body of the Chesapeake Bay the major source of NO_3^-, which occurs in higher concentration than any other form of combined inorganic N, is the Susquehanna River at the head of the Bay. From winter through late spring, there is a consistent inverse linear relationship between NO_3^- concentration and salinity, which definitely implicates freshwater inflow as the primary source of this nutrient. Moreover, the rates at which the biota utilize this nutrient are slow enough to permit NO_3^- to reach the southern end of the Bay during winter and spring. Results of a box model analysis indicate that during spring, when NO_3^- concentrations reach annual maxima in the entire Bay, substantial quantities of N in this form are exported to continental shelf waters (Taft et al. 1978.) During summer, however, the biological consumption of NO_3^- results in low concentrations throughout the Bay, and export is negligible.

Ammonium also reaches highest concentrations in the uppermost portion of the Chesapeake Bay during winter, but for the southern two-thirds of the Bay, there is little seasonality in the distribution of this nutrient. Estimates of NH_4^+ turnover rates and determinations of the rates at which phytoplankton utilize NH_4^+ have led to the conclusion that, unlike NO_3^-, most NH_4^+ in the Bay is produced locally (Carpenter et al. 1969; McCarthy et al. 1975, 1977). Nevertheless, in some areas, such as in the vicinity of Baltimore Harbor and occasionally near the mouth of the Potomac River, anthropogenic sources are evident. The flushing time of the Bay is long relative to the residence time of NH_4^+, hence, the effect of the point source of NH_4^+ is quite local.

Another well-documented, seasonal phenomenon in the Chesapeake Bay is a subpycnocline anoxia that occurs between June and September. The intensity and persistence of this condition are probably influenced by several factors, recently reviewed by Taft et al. (1980). It was concluded that water column respiration is the primary process by which oxygen is depleted during the summer when the pycnocline is well developed. The processes that are responsible for depleting O_2, yield NH_4^+. Hence the NH_4^+ content below the pycnocline is high, relative to that in near-surface waters. For most of the Bay, the upward flux of NH_4^+ across the pycnocline is sufficient to support only a negligible fraction of the N requirement of the photoautotrophs, although on the uppermost part of the Bay this flux can be more significant (Carpenter et al. 1969, Taft et al. 1978).

A less well-documented feature of nutrient distribution patterns in the Chesapeake Bay is the appearance of high NO_2^- concentrations in the late summer to early fall. This was first observed in the open waters of the Bay, south of the confluence of the Patuxent River and north of the Rappahannock River in 1971, and it has been seen regularly since then, during the same period of the year in the mid-Bay region (McCarthy et al. 1977; unpublished data). It is possible that this annual appearance of NO_2^- was missed in earlier studies, some of which reported only the sum of NO_2^- plus NO_3^-.

During the periods of high NO_2^- the concentrations may reach approximately 10 µg atoms $N \cdot liter^{-1}$, and at these times NO_2^- can represent up to 95% of the total nitrogeneous nutrient pool in the near-surface waters (McCarthy et al. 1977). It can be an important nutrient for the phytoplankton (up to 80% of the N ration), with measured turnover times on the order of a few days. Such widespread occurrence of near-surface NO_2^- without an apparent spatial gradient leads to speculation that the origin is within the water column, rather than in the tributaries or shoreline marshes. Nitrate is virtually absent at this time, so it is unlikely that the NO_2^- could be produced via denitrification. Similarly, high concentrations of NO_2^- were also observed above the pycnocline in the York River, immediately prior to a destratification event associated with a spring tide (Webb and D'Elia 1980). In this instance also, NO_3^- apparently was not available to serve as the substrate for denitrification.

The purpose of the present study is to identify the origin of the NO_2^- that appears periodically in the open waters of the Chesapeake Bay. We hypothesize that the bacterial oxidation of NH_4^+ to NO_2^- or primary nitrification, is the most likely source, and when it occurs at rates in excess of those for phytoplankton and bacterial consumption, NO_2^- accumulates in near-surface waters. Bacterial nitrification is probably stimulated when physical processes diminish the strength of the pycnocline, increasing the mixing rate of near-surface waters (high O_2 and low N) with deeper waters (low O_2 and high NH_4^+).

The relationship between rates of primary nitrification and NH_4^+ concentration for marine bacterial isolates capable of oxidizing NH_4^+ have been studied by Watson (1965) and Carlucci and Strickland (1968). The response varied greatly among the clones, and for some, the NH_4^+ concentrations typical of deep Chesapeake Bay waters in summer (10-20 μg atoms $N \cdot liter^{-1}$) would be sufficient to permit near maximal activity. Nitrifiers are obligate aerobes, but for some isolates O_2 concentrations as low as 1% saturation are sufficient to sustain measurable rates of NH_4^+

oxidation (Gunderson 1966; Carlucci and McNally 1969). If, for the common Chesapeake Bay nitrifiers, the requirement for O_2 is greater for secondary nitrification, as it is for Nitrobacter oxidizing NO_2^- compared to Nitrosomonas oxidizing NH_4^+ (Painter 1970), or if the requirement of the indigenous secondary nitrifiers for NO_2^- is greater than that of typical concentrations for oxygenated waters, then NO_2^- could be expected to accumulate. Autotrophic nitrifiers capable of oxidizing NH_4^+ have also been shown to produce N_2O (Yoshida and Alexander 1970), and the yield of N_2O relative to NO_2^- is highest at low concentrations of O_2 (Goreau et al. 1980).

Another source of NO_2^- in marine waters is its release by phytoplankton that are growing on NO_3^- (Vacarro and Ryther 1960; Carlucci et al. 1970; Kiefer et al. 1976). This process is most evident at low light levels, but it is probably not significant during autumn in the Chesapeake Bay because NO_3^- typically is not present in detectable concentrations.

We gratefully acknowledge K.L. Webb, L.W. Haas, C.F. D'Elia and R. Loftus for their assistance in several aspects of the field work.

METHODS

This study consisted of two phases. From 29 August through 1 September 1980, an anchor station at the mouth of the York River was occupied by the R/V RETRIEVER (station YRM 3 in Fig. 1). This period was chosen because it closely followed a spring tide (28 August), and our experiments were part of a destratification study undertaken by K.L. Webb and L.W. Haas of the Virginia Institute of Marine Science, and C.F. D'Elia of the Chesapeake Biological Laboratory. We determined the rates of NH_4^+ uptake and remineralization, the rates of NO_2^- uptake and formation from NH_4^+ and concentrations of N_2O and CH_4, in addition to those of NH_4^+, NO_2^-, and NO_3^-.

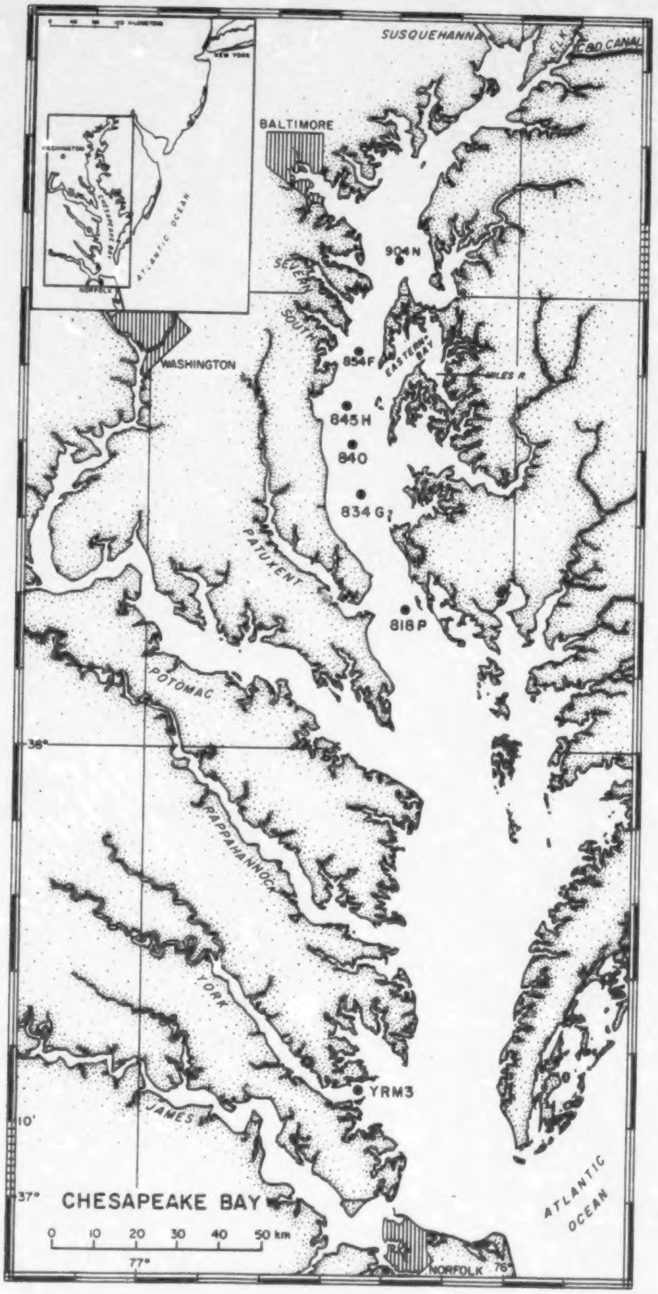

Figure 1: Sampling locations in the Chesapeake Bay and York River.

Water samples for routine chemical analysis of nitrogenous nutrients and dissolved gases were taken at 2 meter intervals using a submersible pump. The water column depth was 18 meters.

The second phase, on 1 and 2 October 1980, aboard the R/V WARFIELD, consisted of an intensive examination of the transformations among the pools of nitrogenous nutrients and particulate N, in the open waters of the Chesapeake Bay at stations between the Patuxent River (818P) and South River (854F) (Fig. 1). This portion of the study was initiated when high NO_2^- concentrations were reported in the near-surface waters of the Bay. We measured the fluxes of N among the NH_4^+, NO_2^-, NO_3^-, and PN pools at three depths in the water column.

On both cruises, sample water for ^{15}N experiments was obtained by repeatedly casting a 3-liter Niskin bottle and draining its contents through a 35 µm Nitex mesh to fill a 20-liter polyethylene carboy. A sample from the carboy was reserved for analysis of initial levels of NO_2^-, NO_3^-, and NH_4^+ (methods as described by Strickland and Parsons 1972, and modified by McCarthy et al. 1977). On the basis of the nutrient concentrations, appropriate amounts of ^{15}N substrates (typically 10% of ambient N, but not less than 0.05 µg atoms $^{15}N \cdot liter^{-1}$) were added to sample volumes contained in 2.75-liter polycarbonate bottles. These were then placed in an on-deck plexiglass tank supplied with flowing near-surface sea water for temperature regulation. The bottles were shaded with neutral density screens or black plastic to simulate in situ light conditions.

Between 2 and 2.5 hours after inoculation with the labeled nutrient, the incubations were terminated by filtration through combusted (60 min at 500°C) Whatman GF/C glass fiber filters. The filters were dried at 60°C and retained for mass spectrographic analysis to determine the rate of incorporation of the label by the particulate fraction of the sample (McCarthy et al. 1977). The quantity of particulate nitrogen was determined manometrically in the mass spectrometer inlet system.

Aliquots of the filtrate from each incubation bottle were reserved for determination of the final concentrations of NO_2^-, NO_3^-, and NH_4^+, and for extraction of the ^{15}N label in the dissolved fraction by the methods outlined below.

Ammonium was recovered from the dissolved fraction by the distillation method described by Glibert et al. (in press). The method used to recover NO_2^- was similar to that first described by Wada and Hattori (1972), and modified by Schell (1978), Olson (1981) and Hashimoto (1981). For samples containing less than about 1 µg atom of NO_2^- N·liter^{-1}, carrier NO_2^- was added in order to provide enough material for the mass spectrographic analysis.

The NO_2^- extraction protocol used in this study was as follows: to a teflon separatory funnel containing 300 ml of the filtrate at room temperature, 5 ml of an analine solution (2.5 grams of analine sulfate in 500 ml 2N HCL) was added and allowed to react for 5 minutes with frequent agitation. Then 5 ml of a β-napthol solution (2.5 grams of 2-napthol (beta) in 500 ml 3N NAOH) was added and allowed to react for about 5 minutes with occasional agitation, followed by a 5 ml addition of 2-M phosphoric acid. The dye thus formed was extracted from the sample by vigorous agitation with three 10 ml aliquots of 1,1,1-trichloroethane. The aqueous fraction having been discarded, the combined extracts were then returned to the separatory funnel and washed with two 100 ml aliquots of 10% HCL, followed by two 100 ml aliquots of distilled water. The extract volume was then reduced in a glass beaker on a hot plate to about two ml, absorbed on a combusted Whatman GF/C glass fiber filter, and air-dried. These filters were stored in glass scintilation vials and subsequently treated in the same manner as the particulate samples for mass spectrographic analysis.

Nitrate was recovered in the same manner, after having been reduced to NO_2^-, by using a peristaltic pump to force the filtrate through a 5 X 100 mm copper/cadmium column at a rate of 8 ml/minute, resulting in a 95% reduction efficiency.

In testing the efficiency of the extraction procedure, we found that the rate of aniline complex formation is temperature sensitive, taking an excessively long time at temperatures less than about 20°C. If this fact is not taken into consideration, incomplete recovery of NO_2^- can result. Glass vials were used for storing the extraction filters because other materials tested (polyethylene and glassine) absorbed the dye.

Simultaneous determinations of the flux of the ^{15}N label from NH_4^+ to NO_2^-, and the evolution of N_2O, were made with aliquots of the mixed carboy sample incubated in 500 ml Vycor Erlenmeyer flasks. They were filled to within 2 cm of the top and $^{15}NH_4^+$ was added. The amount of ^{15}N added was calculated to enrich the sample to about 10 atom percent. The flask was purged (300 ml·minute^{-1}) with helium containing 20% O_2, 300 ppm CO_2, and <10 ppb N_2O, using a gas bubbling frit. After 10 minutes the flow was shut off and the gas line was disconnected. The flask was then topped off with previously purged sample water and sealed with a silicone rubber stopper. The flasks were incubated on deck, in the dark, at near-surface temperatures for 7-8 hours.

At the end of the incubation period, 60 ml of the sample was drained from the flasks into BOD bottles for N_2O analysis. The remaining liquid was analyzed for nitrogenous nutrients, and the NO_2^- and NH_4^+ were recovered for the determination of ^{15}N enrichment. Experiments to determine the N_2O

levels at time-zero were performed on samples without ^{15}N enrichment.

In calculating uptake, remineralization, and nitrogen transformation rates with ^{15}N labeled substrates, we used the mathematical formulations described by Glibert et al. (in press).

Production of NO_2^- during nitrification was measured by incubating Bay water in specially constructed one-liter glass syringes fitted with teflon plungers, which permitted samples to be withdrawn from the Niskin bottle without entrapping air bubbles. The syringes were incubated in the dark at near-surface seawater temperature. Periodically over a three-day period, aliquots were removed from the syringes for analyses of O_2 (Strickland and Parsons 1972), N_2O (Kaplan et al. 1978) and nitrogenous nutrients. The samples for nutrient analyses were filtered through Whatman GF/C glass fiber filters, placed into polyethylene bottles, and frozen. They were assayed using automated methods (Strickland and Parsons 1972), one to two weeks later.

On both cruises, samples for CH_4 and N_2O analyses were placed in 60 ml BOD bottles with one ml of a saturated $HgCl_2$ solution added as a preservative. Dissolved N_2O was assayed by electron-capture detector gas chromatography, as described earlier (Kaplan et al. 1978). Samples for CH_4 were analyzed using an identical static equilibrium method with a Perkin-Elmer 3920B gas chromatograph that contained a flame ionization detector. We used a column of molecular sieve 5A, operated at a temperature of 60°C.

Results

York River

On 29 August there was distinct density stratification in the water column (i.e., delta sigma-t surface to bottom of approximately 1.6), but by 30 August this difference was <0.5. Therefore, for the purposes of this study, we consider that the water column destratified on or about 30 August.

Nutrient concentrations through the study period are plotted in Fig. 2 (note the log scale on the ordinate). Ammonium at both 3 m and 12 m shows a strong oscillation, with the minimum values occurring at high slack tide and the maximum values at low slack tide. The deeper samples generally contained more NH_4^+ and the concentrations at both depths declined over the course of the study. Nitrite concentrations oscillated with a similar period but were generally low, ie. < 0.4 µg atoms·liter^{-1}. Initially the deeper NO_2^- values were higher, but in the entire data set, there is no consistent difference between the two depths. By the last day of the study, NO_2^- had increased in the water column, but the high near-surface NO_2^- values, previously observed during a similar tidal destratification in the York River (Webb and D'Elia 1980), were not seen during this study.

Uptake and remineralization rates for NH_4^+ are plotted in Fig. 3. Prior to the destratification of the water column, uptake of NH_4^+ had a diel pattern, with the lowest values at night and the highest values during the day. Ammonium remineralization during this period followed the same pattern, except for the 12 m sample at 2000 h on 29 August. After destratification, the diel pattern persisted, but the nighttime rates of both NH_4^+ uptake and remineralization were higher than they had been previously.

Nitrite uptake and transformation of NH_4^+ to NO_2^- are plotted in Fig. 4. (Note that NO_2^- uptake experiments were not started until 31 August.) The transformaton of NH_4^+ to NO_2^- occurred sporadically (with respect to time and tide), but frequently at high rates. A nadir lasting about 12 hours followed the destratification of the estuary, and subsequent rates, especially at 3 m,

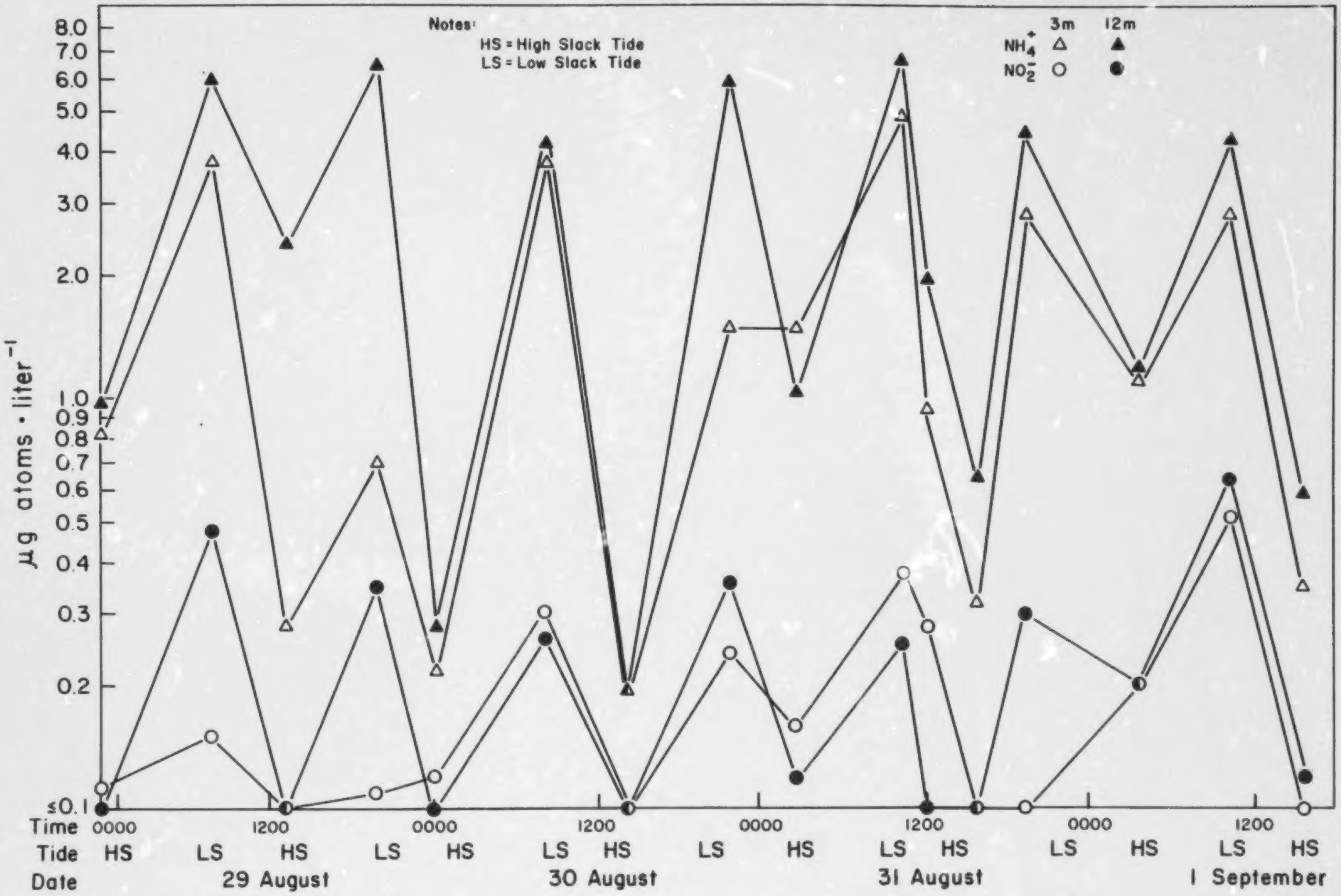

Figure 2: Concentrations of nitrite and ammonium at the York River station.

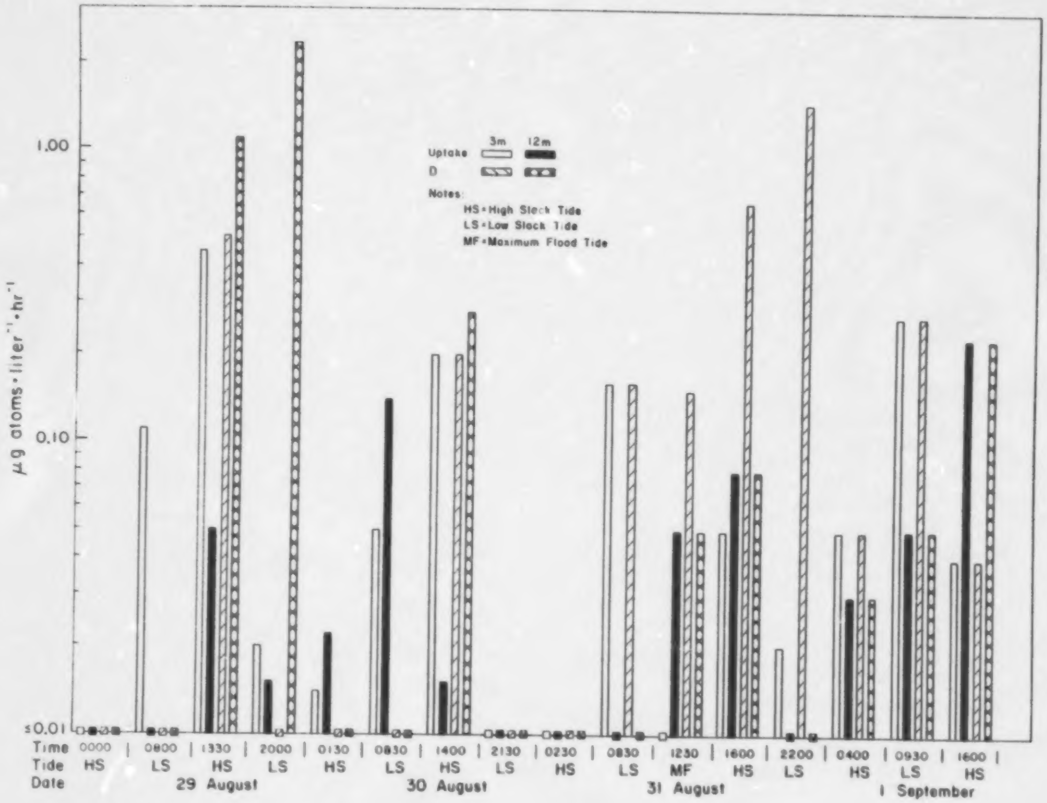

Figure 3: Uptake and remineralization rates for ammonium at the York River station.

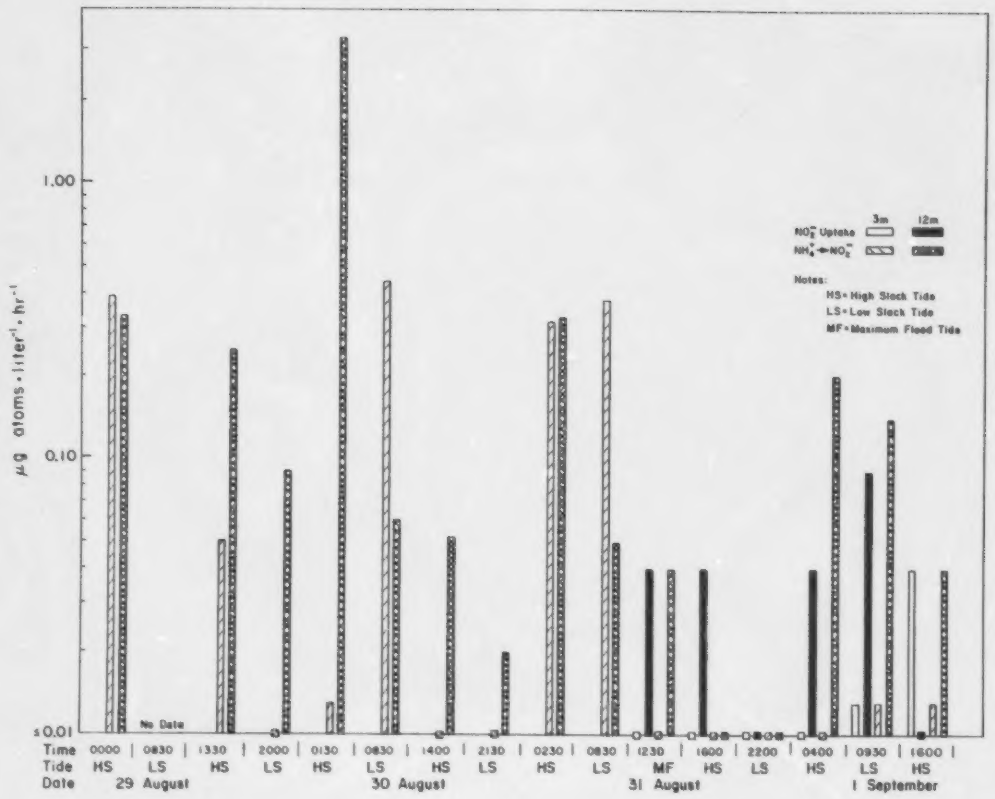

Figure 4: Uptake of nitrite and production of nitrite from ammonium at the York River station.

remained lower than those measured prior to destratification. Of the two depths sampled, uptake of NO_2^- could be detected only in the 12 m daytime samples. On 2 September, however, the rate increased markedly at 3 m and became undetectable at 12 m. This increase in the NO_2^- uptake rate at 3 m coincided with the moderate increase in ambient NO_2^- (Fig. 2).

Nitrate concentrations and uptake (not plotted) were determined only for the last part of the study. Like NO_2^-, NO_3^- concentrations were also very low until 1 September at 1600 h, when the concentration was 2.0 µg atoms·liter^{-1} or approximately five times its level at any time during the previous 30 hours. At this time, the rate of NO_3^- uptake also reached a maximum and was similar in magnitude to the rate of NH_4^+ uptake. The NH_4^+ concentration was 0.35 µg atoms·liter^{-1}.

In all water samples taken during the destratification event, the distribution of CH_4 differed markedly from that of N_2O. There was a strong positive relationship ($r=0.92$) between dissolved CH_4 and sigma-t for all samples taken at low slack tide (Fig. 5). Methane is an unequivocal tracer of anoxic metabolism, and its distribution in the water column suggests that methane is produced at or near the bottom. High tide samples show more scatter ($r=0.66$) and lower absolute levels although there is also an apparent CH_4 source in denser Bay water, probably associated with the sediments. Surface concentrations of CH_4 remained fairly constant regardless of tidal stage. Thus, the data sets tend to converge at a sigma-t of 13.5 (Fig. 5). This value characterizes water above the pycnocline (0-6 m).

The distribution of NH_4^+ shows the same positive relationship to density as CH_4. Low tide NH_4^+ concentrations are typically 5-6 times greater than high tide values. Although we have fewer data for NH_4^+, the similarity to the CH_4 plot of Fig. 5 suggests that the NH_4^+ source is also at or near the

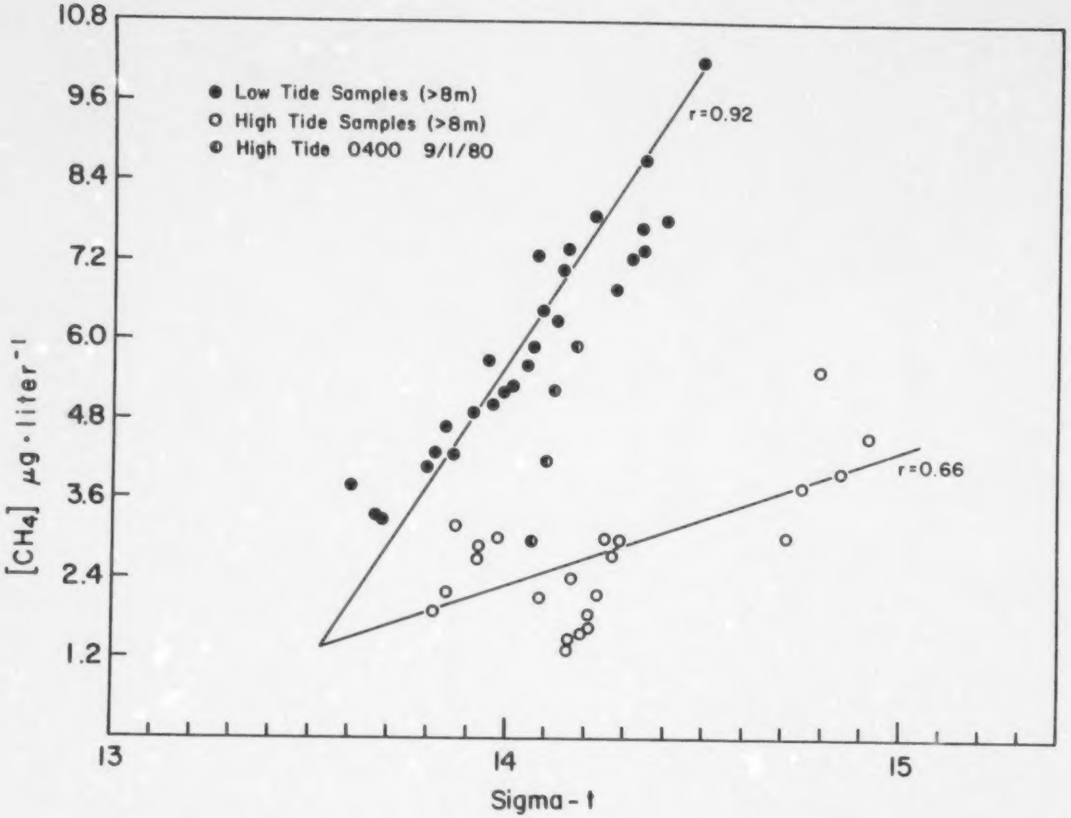

Figure 5: Relationship between methane and sigma-t during tidal destratification at the York River station.

bottom. A more extensive data set (Webb and D'Elia, personal communication) supports this conclusion.

The distribution of N_2O is distinctly different from those for both CH_4 and NH_4^+ (Fig.6), having no relation to density, regardless of tidal stage. Nitrite distribution was also without an apparent relationship to density, and unlike N_2O, the NO_2^- values tend to be highest during low slack tide (Fig. 2).

Chesapeake Bay

Data provided by J.L. Taft (with the assistance of R. Loftus and C.F. D'Elia) enabled us to time this portion of the study to be coincident with high concentrations of NO_2^- in the northern portion of the Bay. These preliminary observations are summarized in Table 1. The near-surface concentrations were similar to those observed in autumn during previous years, and the high concentrations in deeper water lend support to the hypothesis that NO_2^- is formed within the water column. Immediately prior to this study, NH_4^+ data were available only for 29 September 1980 at station 818 (0.2 μg atoms NH_4^+-N·liter^{-1} at the surface and 16 μg atoms NH_4^+-N·liter^{-1} at 16 m). During September, both high NH_4^+ and low O_2 are typical for deep water in this region of the Bay.

Summary graphs of nutrient and oxygen concentrations, at three stations for the three depths studied, are given in Fig. 7 (note that on one day station 854D was sampled twice: AM and PM); the intermediate depth was chosen to be in the region of strongest density gradient.

At station 854D the NO_2^- concentrations were less, both at near-surface and below the pycnocline, than those observed at this station during the previous week. The deep water was anoxic, although it contained

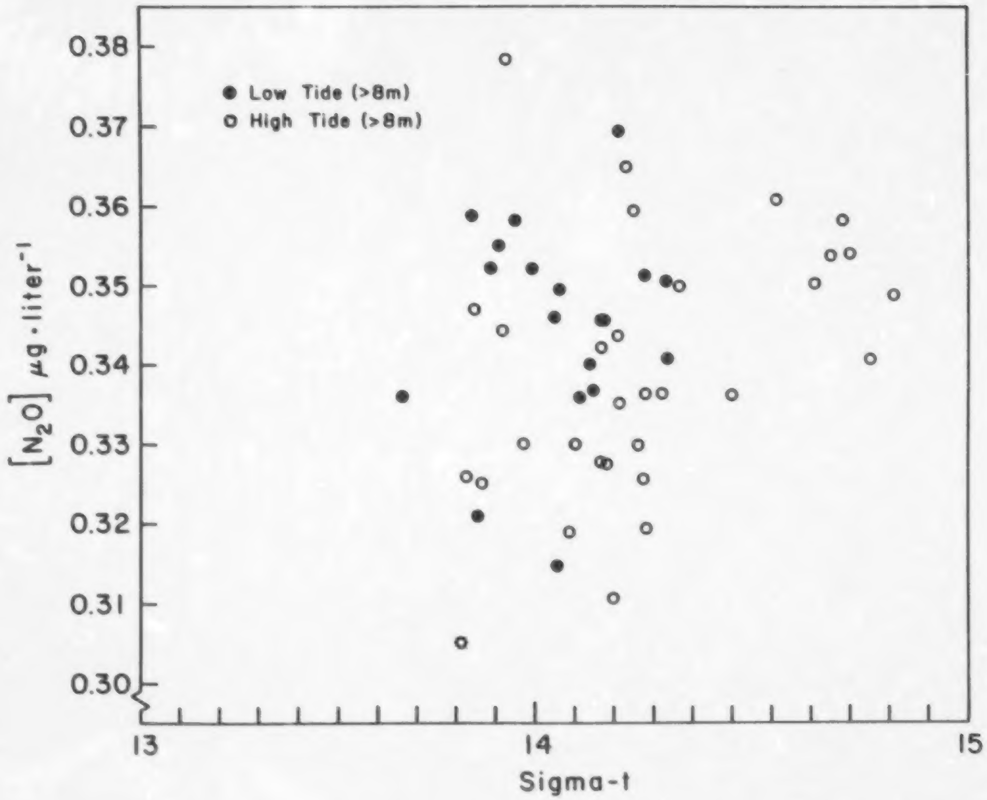

Figure 6: Relationship between nitrous oxide and sigma-t during tidal destratification at the York River station.

Table 1.

Nitrite concentrations reported for the Chesapeake Bay south of Baltimore Harbor and north of Solomons, Md. between 22 and 29 September 1980.

Date	Station	NO_2^- (μg atoms N·liter^{-1}·h^{-1}) Near-Surface	Subpycnocline
22-24 Sept.	854	2.5	4.0
	845	5.0	>6.0
	834	≤1.0	>6.0
	818	≤1.0	>10.0
26 Sept.	904	5.8	
	854	6.4	
	845	6.6	
	840	3.7	
	834	1.9	
	818	1.2	
29 Sept.	818	0.2	10.0

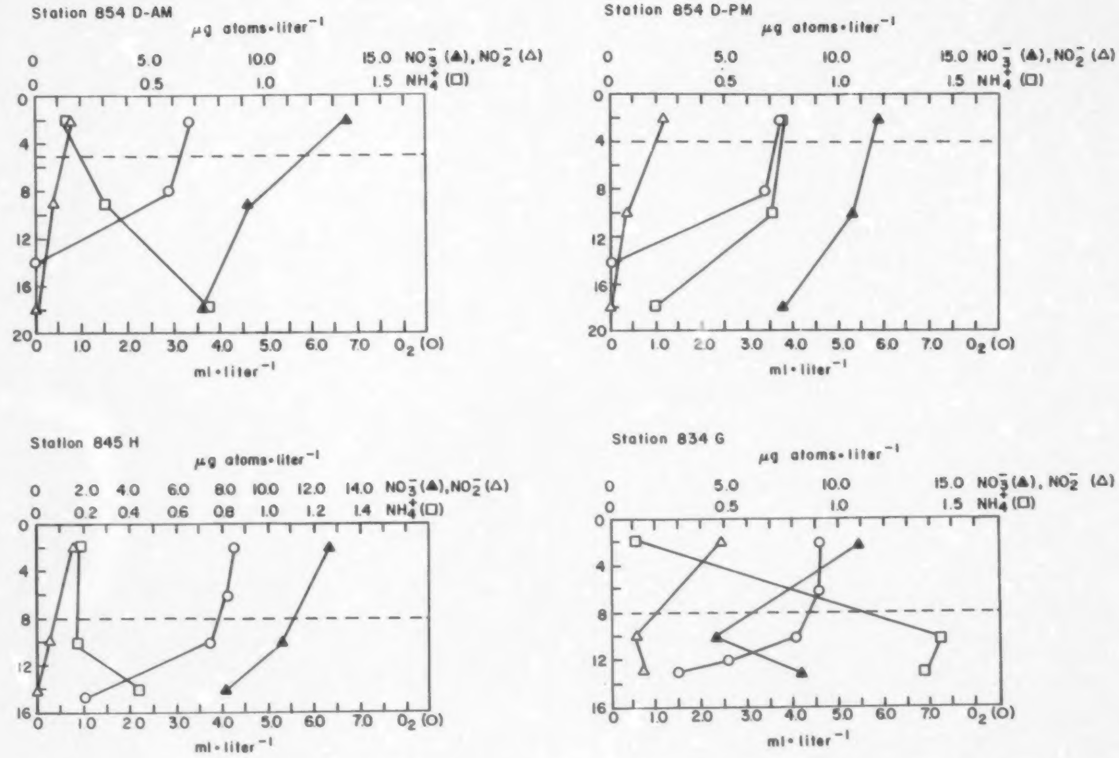

Figure 7: Nitrate, nitrite, and ammonium concentrations at the upper Chesapeake Bay stations.

Dashed line indicates depth of upper mixed layer.

surprisingly little NH_4^+. Of perhaps even greater interest, was the concentration of NO_3^- throughout the water column. Between early morning and afternoon NH_4^+ was the only constituent for which the distribution changed. Differences of this magnitude for NH_4^+ are not unusual at single stations over the course of a day in the Chesapeake Bay (unpublished data). Further south at 845H, the deep water contained O_2, the NH_4^+ concentration was again less than would have been expected, and the NO_2^- concentration was less than had been observed during the previous week (Table 1). Nitrate was again high throughout the water column. At station 834G the deep water contained O_2, and the NH_4^+ concentration was the greatest observed during the study (1.45 µg N·liter^{-1}). The NO_2^- concentration in near-surface waters at station 834G was also the greatest observed (4.90 µg atoms N·liter^{-1}). This pattern of NO_2^- distribution with depth was the opposite of that observed during the previous week (Table 1). Again at station 834G, there was a large quantity of NO_3^- throughout the water column. Only at this station, however, was there an apparent NO_3^- minimum in the region of the pycnocline.

The fluxes from one form of N to another, as determined in the ^{15}N labeling experiments, are presented in Figs. 8, 9, 10, and 11. If all processes were considered and all measurements were accurate, the sums of these rates should be equivalent to changes in pool size over the 2-2.5 h period of the incubation. It is difficult to obtain sufficient precision in the determination of pool size changes over such brief periods, however, with the exception of NO_3^-, which is discussed below, there were no great discrepencies in these comparisons. In considering the rates determined for deeper samples, it should be remembered that except for the syringe incubation experiments our sample collection procedure permitted atmospheric O_2 to enter samples and increase O_2 concentrations in samples that initially were at less than saturation values. For this reason, the rates determined for samples from depths at which O_2 was depleted should be considered as the potential rates at increased concentrations of O_2.

At station 854D - AM (Fig. 8), the dominant flux for the NO_2^- pool was an input from NO_3^- at 2 m. Since these waters were well-oxygenated, we would not have expected denitrification to occur at a significant rate. The same process took place at a lesser rate in the 9 m sample where O_2 was still high. At both 2 m and 9 m, there was evidence that NO_2^- was reduced to NH_4^+. Nitrite formation from NH_4^+ occured at a significant rate only at 9 m. At 18 m the apparent production of NO_2^-, as measured by isotope dilution, was not reflected in any of the sparate source measurments.

The loss of NO_3^- to NO_2^- is also the largest flux from the 2 m NO_3^- pool. Evidence for reduction of NO_3^- also exists in the NO_3^- to NH_4^+ flux; however, the rate of this process for NH_4^+ formation was less than the rate at which NH_4^+ was oxidized to NO_3^-. Again at 9 m, there is evidence for the reduction of NO_3^- to NO_2^-, and evidence both that NO_3^- was reduced to NH_4^+ and that NH_4^+ was oxidized to NO_3^-. At 18 m the largest flux (with O_2 added) was the oxidation of NH_4^+ to NO_3^-.

Other fluxes to and from the NH_4^+ pool include the largest flux to particulate nitrogen (PN) from any substrate throughout the water column, although at 2 m the flux of NO_3^- to PN was greater than that for NH_4^+. At 18 m the large flux into the NH_4^+ pool, as measured by isotope dilution, is not reflected in any of the separate source measurements. It could have arisen from heterotrophic activity metabolizing particulate, or dissolved organic N.

During the afternoon of the same day, many of the fluxes at station 854D (Fig. 9) were similar to those observed during the morning. Some, such as

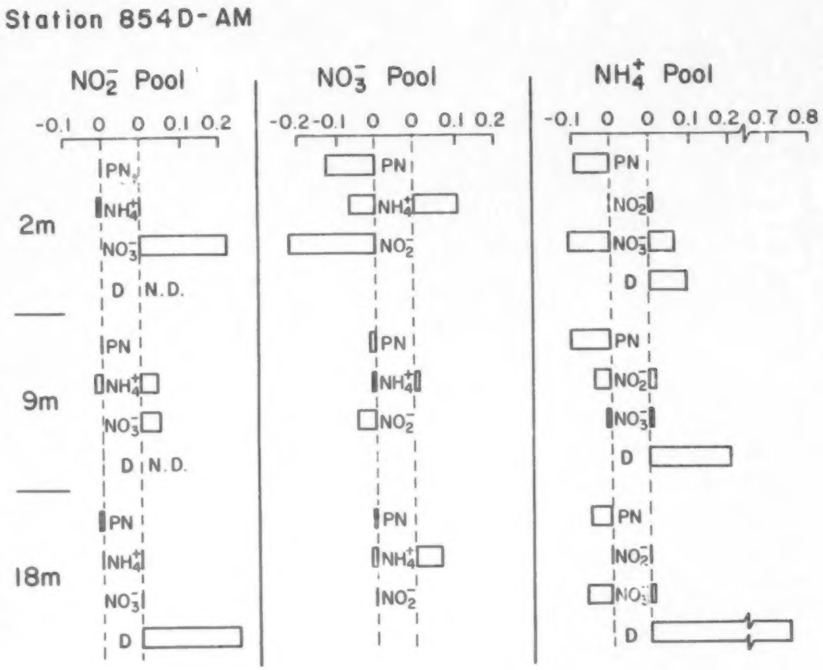

Figure 8: Nitrogen fluxes, as µg atoms N·liter^{-1} determined at station 854D, AM, on October 1, 1980. The forms of nitrogen indicated in colums for each pool represent different sources (+) and sinks (-) for the substrate in the pool. The D categories under NO$_2^-$ and NH$_4^+$ represent fluxes to the NO$_2^-$ and NH$_4^+$ pools, respectively, as measured by isotope dilution.

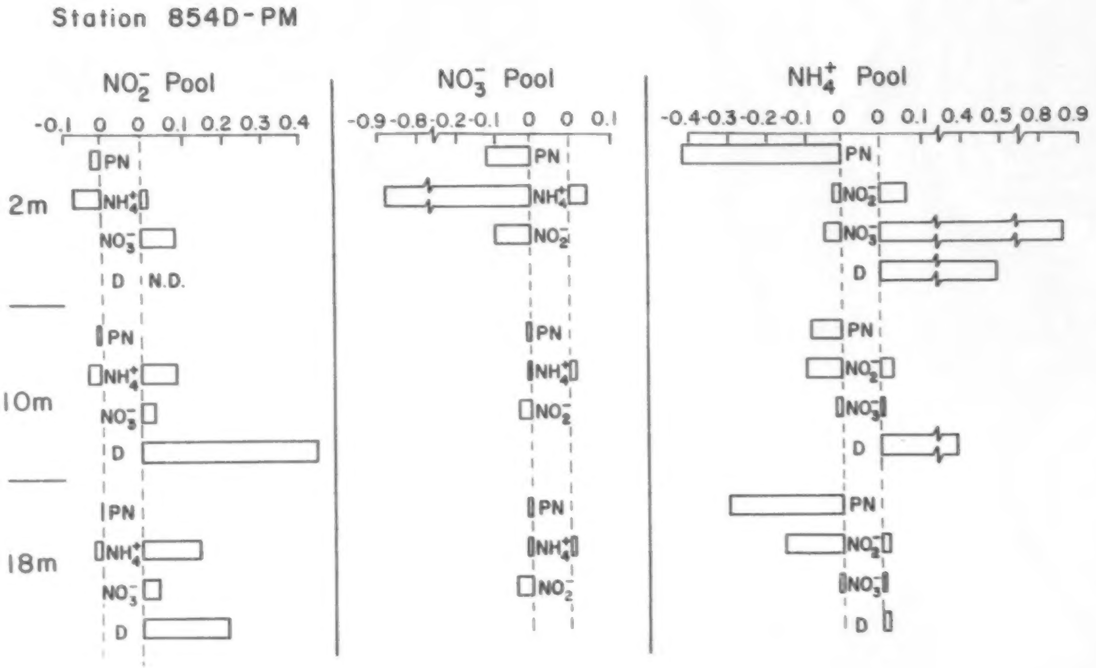

Figure 9: Nitrogen fluxes, as μg atoms N·liter^{-1} determined at station 854D, PM, on October 1, 1980. The forms of nitrogen indicated in columns for each pool represent different sources (+) and sinks (-) for the substrate in the pool. The D categories under NO$_2^-$ and NH$_4^+$ represent fluxes to the NO$_2^-$ and NH$_4^+$ pools, respectively, as measured by isotope dilution.

the fluxes both from NH_4^+ to NO_2^- and NO_2^- to NH_4^+, were greater at 2 m and 18 m. The large NO_2^- flux at 10 m is without an obvious source.

For the NO_3^- pool, the larger rate of reduction to NH_4^+ at 2 m is the most apparent difference between morning and afternoon. The 10 m sample was essentially the same at both times. At 18 m there was a greater rate of reduction of NO_3^- to NO_2^-, and lesser rate of oxidation of NH_4^+ to NO_3^-.

Most conspicuous in the morning-to-afternoon differences for the NH_4^+ pool are the greater afternoon fluxes to PN. The rates of NH_4^+ input, as measured by isotope dilution, were greater at 2 m and less at 18 m than those determined in the morning.

At station 845H (Fig. 10), the 2 m sample had the greatest rate of NO_2^- reduction to NH_4^+. The rate observed at 14 m, although less than at 2 m, was also higher than those seen at station 854D. At each depth, and at an increasing rate with depth, NH_4^+ was oxidized to NO_2^-; however, these rates were always less than those for the reverse process. At both 10 m and 14 m, NO_2^- was oxidized to NO_3^-.

Some of the contribution to the NO_3^- pool at 2 m was from the production from NH_4^+ oxidation; and at 14 m, NH_4^+ oxidation to NO_2^- and NO_3^- occurred at similar rates. The dominant flux for the NO_3^- pool at 10 m and 14 m was the reduction of NO_3^- to NH_4^+, and the rate at 14 m was the highest observed during this study. At 10 m and 14 m, NO_3^- was also reduced to NO_2^- at rates similar to those seen at station 854D.

The fluxes involving NH_4^+ that have already been mentioned dominate the rates of input and loss to the NH_4^+ pool. By comparison, the loss to PN was of relatively minor consequence at 2 m and 10 m.

At station 834G (Fig. 11), the 2 m sample had the lowest rate of NO_2^- reduction to NH_4^+. The dominant NO_2^- flux at this depth was the reduction to

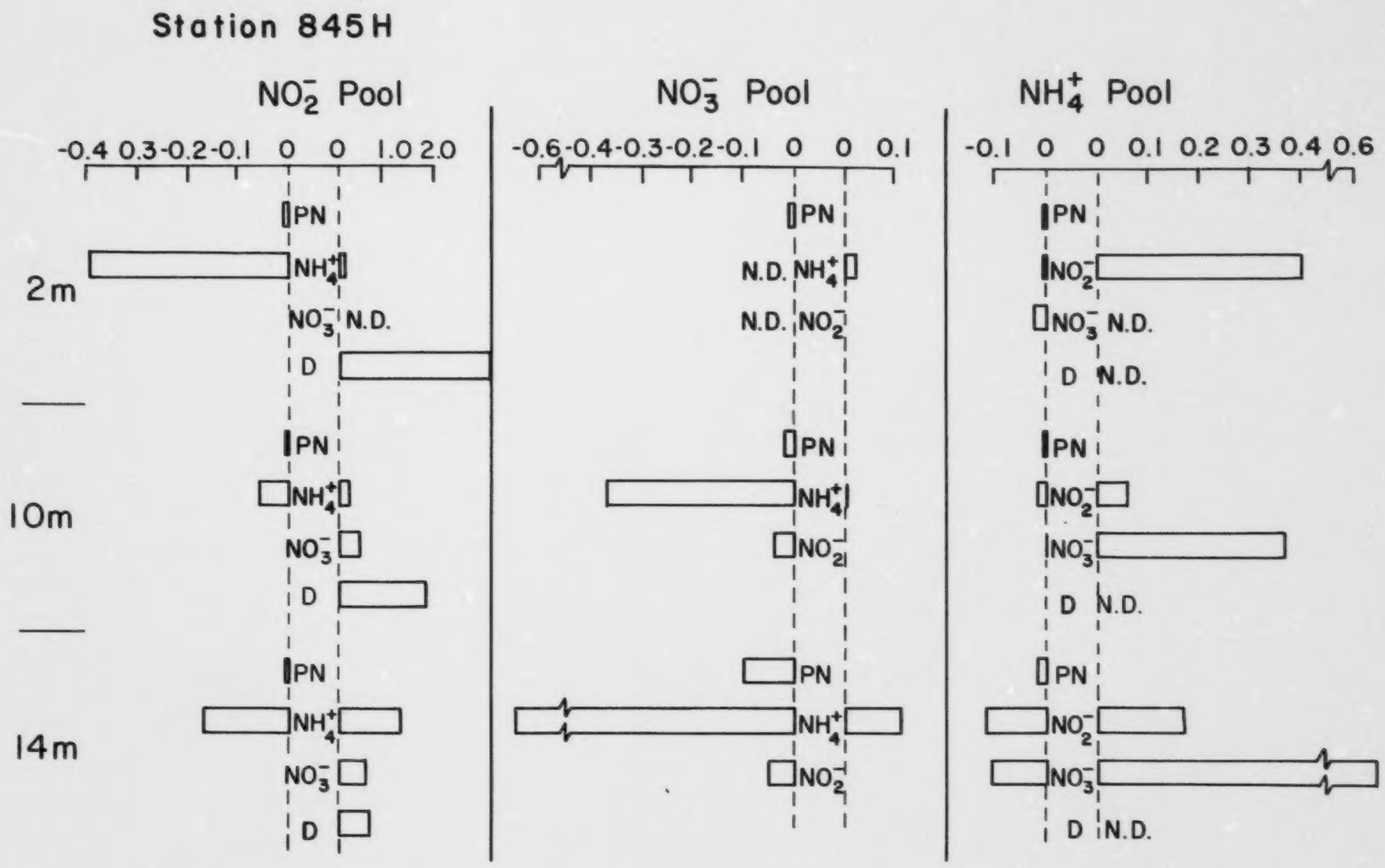

Figure 10: Nitrogen fluxes, as µg atoms N·liter^{-1}, determined at station 845H on October 2, 1980.

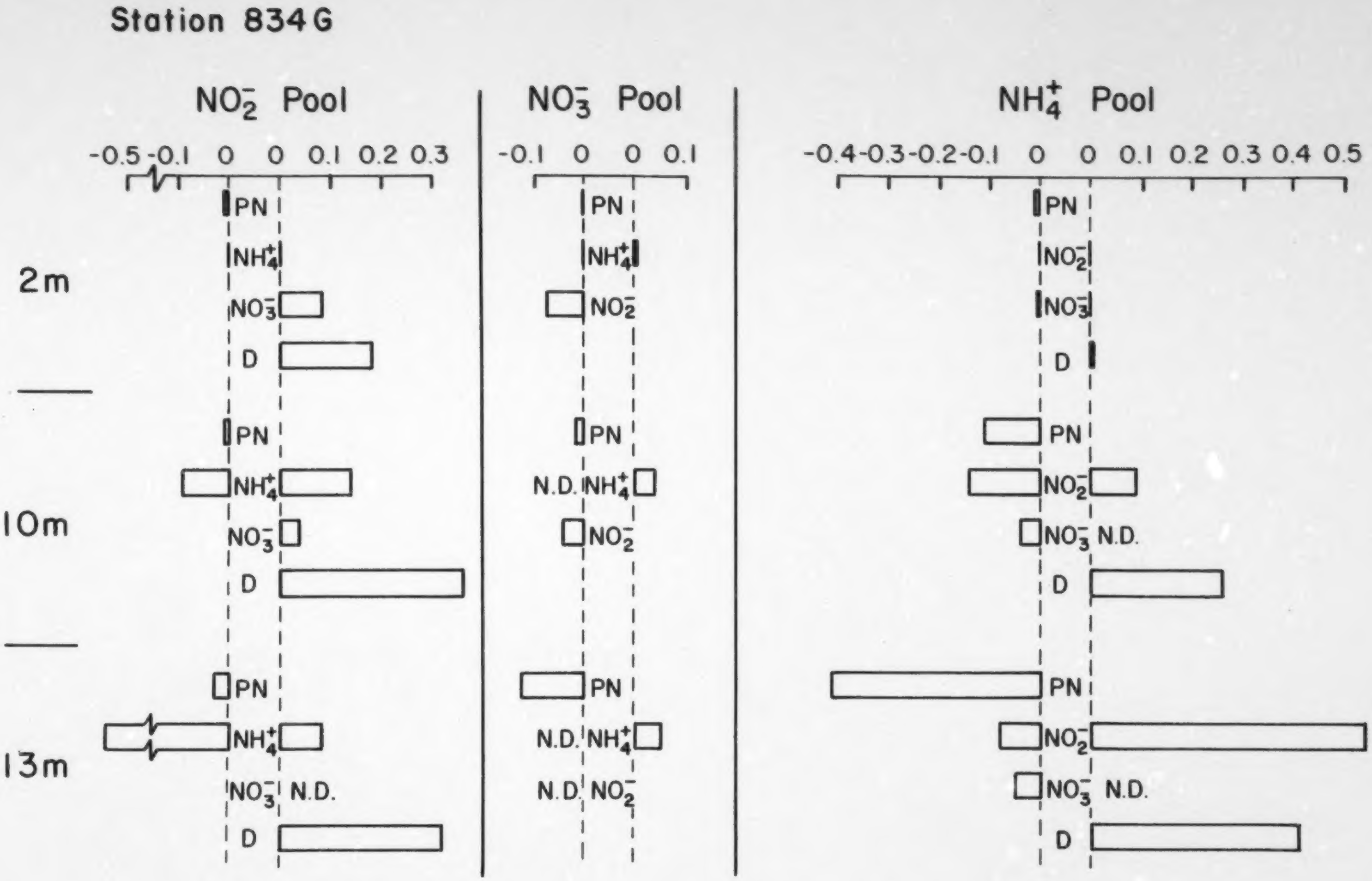

Figure 11: Nitrogen fluxes, as µg atoms N·liter^{-1}, determined at station 834G on October 2, 1980.

NO_3^-. The 10 m sample had fluxes in both directions between the NO_2^- and NH_4^+ pools.The 13 m sample had the highest measured reduction from NO_2^- to NH_4^+.

For the NO_3^- pool, several fluxes gave insignificant rates, but there was measurable reduction of NO_3^- to NO_2^- at 2 m and 10 m, and from the oxidation from NH_4^+ to NO_3^- at all depths. For the NH_4^+ pool all fluxes were slow at 2 m, although those involving NO_2^- and NO_3^- rival in magnitude the flux to PN. At 10 m and 12 m all fluxes were greater, and the rate of NH_4^+ incorporation into PN at 13 m was the greatest seen from this flux.

The data presented in Table 2 are the result of the first field experiments to determine a net yield of N_2O during nitrification in aquatic systems. Yields average 0.6%, which is within the range of results reported both from pure cultures of marine nitrifying bacteria (Goreau et al. 1980) and from inferences made using bulk concentration of N_2O, O_2, and nitrogenous nutrients (Elkins et al. 1978; Cohen and Gordon 1979; Kaplan et al. unpubl).

It is important to note that the Vycor-yield experiments were performed using a gassing mixture that contained 20% oxygen. Considering (1) that the ambient O_2 concentrations were 30-50% undersaturated with respect to the atmosphere, and (2) that O_2 exerts a strong effect on N_2O production during nitrification (Goreau et al. 1980), in situ yields may have been somewhat higher. It may be only coincidental that there was such a close correspondence between rates of NO_3^- and NO_2^--N production in labeled (Vycor) and unlabeled (syringe) experiments. There is no a priori reason to expect this, because both NO_3^- and NO_2^- were also consumed in these samples.

Discussion

The results of the ^{15}N incubation studies clearly indicate the potential

Table 2.

Production of N_2O, NO_3^-, and NO_2^- (ng atoms $N \cdot liter^{-1} \cdot h^{-1}$)and fractions of total as N_2O. Except where noted, experiments used pooled carboy samples.

Syringe Experiments

Station	Depth (m)	N_2O Production	NO_3^- & NO_2^- Production	N_2O Yield
854D - PM	9	0.303	17.9	0.0169
834G	13*	0.193	54.3	0.0036
	13**	0.118	46.6	0.0025

Vycor Erlenmeyer Experiments

Station	Depth (m)	N_2O Production	$^{15}NH_4^+$ flux to NO_2^- and NO_3^-	NO_2^- Yield
854D - PM	2	0.360	51.0	0.0070
	9	0.207	57.0	0.0036
834G	13	0.126	53.0	0.0023

* This sample contained 5.5 ppmO_2 (for comparison with the next sample).

** This sample was taken directly from Van Dorn bottle and contained 3.0 ppmO_2.

for nitrification during the period of our study, and it seems likely that this process is responsible for the autumn increase in NO_2^- in near-surface waters of the Chesapeake Bay. October 1980 appears to have been the first time that high levels of NO_3^- were also observed in this region of the Chesapeake Bay in autumn, and the potential for secondary nitrification was also confirmed by our experimental results. The findings show that NO_2^- and NO_3^- can be formed in the water column of the Bay, and if this source of NO_3^- is significant relative to riverine input, it will be more difficult to budget N in this water body than has been previously presumed. The NH_4^+ that accumulates below the pycnocline during spring and summer, when the deep anoxia develops, apparently can be oxidized to NO_2^- and/or NO_3^- following disruption of the pycnocline, such as with late summer or early fall storms. Local or annual variations in these basic conditions will determine whether or not either NO_2^- or NO_3^- is produced rapidly enough to accumulate in the water column. If sufficient NH_4^+ is also mixed into, or produced within, the near-surface waters to meet the needs of the phytoplankton, the accumulation of NO_2^- and/or NO_3^- will be more likely than if NH_4^+ is kept at low concentrations by the various processes that consume NH_4^+.

Evidence that the capacity to reduce both NO_3^- and NO_2^- to NH_4^+ exists throughout the water column during this period is puzzling. At station 854D, the high NO_3^- in anoxic deep water is likely to be reduced to either NH_4^+ or nitrogenous gasses, but the rates of NO_3^- reduction to NO_2^- and NH_4^+ were relatively low at 18 m. Conceivably, denitrification was occurring in this sample, but we have no measure of the production of $^{15}N_2$. The greatest potential for reducing NO_3^- and NO_2^- was observed at 14 m at station 845H (Fig. 10). This rate would permit a turnover time of the deep NO_3^- (Fig. 7) in less than 13 h. The greatest rate of NO_2^- reduction was observed at 13 m at station 834G. This rate (Fig. 11) would permit a turnover time of the deep NO_2^- (Fig. 7) in less than 3 h.

Other data indicate that nitrogenous gasses are not the sole end product of NO_3^- reduction in coastal regions. In studies of coastal and estuarine sediments (Koike and Hattori 1978a; Sorensen 1978; Buresh and Patrick 1981), up to 50% of the ^{15}N label added as NO_2^- and NO_3^- was recovered as NH_4^+. Moreover, it has been demonstrated that losses of NO_3^- can occur under oxygenated conditions (Focht and Verstraete 1977; Koike and Hattori 1978b). In the case of denitrification, this has been attributed to the existence of anoxic microzones associated with particles for which the consumption of O_2 exceeds the rate of supply from the bulk medium (Greenwood 1961). It is not known whether dissimilatory NO_3^- reduction to NH_4^+ also occurs in association with anoxic microzones. However, the admixture of near-surface and deep waters can transport particulate material to the oxygenated region above the pycnocline. This could permit the oxidation of NH_4^+ to NO_2^- and/or NO_3^-, and the reduction of NO_3^- and/or NO_2^- to NH_4^+ to take place simultaneously in the oxygenated near-surface waters.

Recent interest in the distribution of N_2O in the environment (Cohen and Gordon 1978; 1979; Elkins et al. 1978) was prompted by its importance in the chemistry of the stratosphere (Nicolet 1975). As a result, there is now a fairly extensive body of work on the biogeochemistry of this gas (see review by Hahn and Junge 1976). In aquatic systems, where the oxygen concentration is greater than a few tenths mg $O_2 \cdot liter^{-1}$, nitrification appears to be a major source of N_2O (Goreau et al. 1980; Cohen and Gordon 1979; Elkins et al. 1978; McElroy et al. 1978).

A key question concerns the importance of the water column relative to the sediments in the production of N_2O. The distribution of CH_4 and N_2O at the York River station is useful in this regard. Based on the profiles of salinity and sigma-t, the water column was vertically homogeneous after 29 August with little difference in these properties between high and low tides. However, the source of CH_4 (Fig. 5) and NH_4^+ was strong enough to counteract mixing processes that maintained this well mixed state: there was always more CH_4 and NH_4^+ in the bottom water. Conversely, the apparent lack of any relationship between either N_2O (Fig. 6), or NO_2^-, and density strongly suggests a nonsedimentary source for these compounds.

The amount of dissolved N_2O in the upper six meters of the water column remained virtually constant throughout the study period, averaging 91 ± 2.6 μg atoms $N_2O\text{-}N \cdot m^{-2}$ for all low tides, and 89 ± 1.3 μg atoms $N_2O\text{-}N \cdot m^{-2}$ for all high tides. The surface waters of the York River station were supersaturated with respect to atmospheric N_2O levels (300 ppb) by a factor of 1.15 - 1.20. Therefore, we postulate either a diffuse source of N_2O and NO_2^- in the river water, or advection of water enriched with N_2O and NO_2^- from nearby wetlands. With the current information, the two alternatives cannot be distinguished conclusively. However, atmospheric loss of N_2O from the surface waters can be calculated, and the steady state nitrification rate needed to support this flux can be estimated.

The thin-film model of Broecker and Peng (1974) is:

$$F = \frac{D}{Z} (N_2O \text{ sample} - N_2O \text{ air, or } \Delta N_2O)$$

where:

D = molecular diffusivity of N_2O (2.1×10^{-5} $cm^{-2} \cdot sec^{-1}$)

Z = stagnant boundary layer thickness (microns)

The critical factor in the model is Z, which is strongly dependent on wind speed (Emerson 1975). Wind speed data were obtained from the National Weather Service, and interpolation of Emerson's (1975) wind speed/Z relationship gives a Z of 150 μm.

Given a ΔN_2O of 0.060 μg $N_2O \cdot liter^{-1}$, the flux integrated over the top meter is 0.343 ng atoms N_2O-$N \cdot liter^{-1} \cdot hr$. If between 0.2 and 0.7% of the products of nitrification (NO_2^--N + NO_3^--N) are released as N_2O-N (See Table 2 and Goreau et al. 1980; Cohen and Gordon 1978), the production of oxidized N in the York River mouth would be between 0.049 and 0.171μg atoms $N \cdot liter^{-1} \cdot hr$. Measured rates vary greatly (Fig. 4), and their range includes the values calculated above, although frequently they were higher than this estimated rate of production.

The results presented here support the view that nitrification may be a major source of N_2O in many aquatic environments. The yield of N_2O during nitrification is dependent upon several factors, including oxygen, temperature, and substrate concentration. Estimates of N_2O yield during nitrification (Cohen and Gordon 1979; Elkins et al. 1978; Kaplan et al. in prep.), based on changes in N_2O, O_2, and nutrient concentrations, range from 0.1 to 0.3% over a wide variety of temperatures and dissolved inorganic nitrogen concentrations. This relative consistency is reflected in the measured yield ratios for laboratory experiments. Under oxygen conditions similar to those of our field data (Fig. 7), laboratory yields averaged 0.3 - 0.5% (Goreau et al. 1980). When the effect of environmental factors on N_2O yield is determined more precisely, it may be possible to accurately estimate rates of nitrification in natural waters by measuring only the production of N_2O.

If anthropogenic sources of nutrients to the Chesapeake Bay result in increased productivity, which in turn leads to more widespread anoxia in the deep waters of the Bay, then the autumn occurrence of high NO_2^- concentrations discussed above may be expected to increase in intensity and duration. In this sense, the production and consumption of NO_2^- and the production of N_2O are both sensitive indicators of the effects of anoxia on N cycling in the Chesapeake Bay.

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CHAPTER VII

Nutrient production and transport in the anoxic deep water of the Potomac River estuary.[1]

Jerome Gavis

and

Jay L. Taft

ABSTRACT

Concentrations of dissolved orthophosphate and ammonia determined during a 43 hr period in the lower Potomac River estuary varied with depth and with time at any depth, but were independent oc time when expressed as functions of salinity. The concentration/salinity profiles in the anoxic deep water could be described by the solution of a differential equation, which was obtained by transformation of the partial differential equations for transport of salt and of a non-conservative nutrient. The solution was expressed in terms of a dimensionless parameter, Φ, equivalent to the ratio of a characteristic nutrient production rate to a characteristic nutrient diffusion rate. Estimates of Φ, obtained by means of non-linear parameter analysis, were 1.13 for dissolved orthophosphate and 1.03 for ammonia. This means that about half of the nutrients in the anoxic deep water were produced in the water by anaerobic mineralization of detritus, and half was transported into the water with salt from higher salinity water down the estuary. Any nutrient flux from the bottom sediment was too small to be discerned relative to the nutrient produced in and transported through the water. The ammonia/dissolved orthophosphate concentration ratio in the anoxic water was 7-8, indicating that, because denitrification could not occur, a smaller fraction of bound amino-nitrogen than of bound phosphate was released by mineralization of detritus as it settled in the anoxic water.

In summer the deep water of a stratified coastal plain estuary may become anoxic. Concentrations of the nutrients ammonia and dissolved orthophosphate are usually much higher in the anoxic water than in the oxygenated water above. The nutrients may enter the anoxic water from the sediment at the bottom, diffusing from and through the reduced sediment surface. They may be transported with salt from higher salinity water lower down the estuary. They may also appear as a result of anaerobic mineralization in the water itself of detrital matter settling from above.

The nutrients can be mixed into the surface water during destratification events. They can also be transported by turbulent diffusion into the surface water while stratification persists. That part of the nutrients that reaches the euphotic zone may be then consumed by plants and contribute to productivity and to an increase in standing crop.

It is necessary here to distinguish between mineralization occurring in oxygenated water and at an underlying oxidized sediment surface from that occurring in anoxic water and at a reduced sediment surface. When oxygen is available, aerobic mineralization takes place, more in the upper layers of the sediment than in the overlying water (Nixon, 1979; Boynton et al., 1980). Nixon (1979) has emphasized that nutrients thus released are recycled nutrients and not inputs to the estuarine system.

When the deep water is anoxic, the surface layers of the sediment become permeable to dissolved orthophosphate which may then diffuse along with ammonia from within the sediment as well as from its surface into the water above. Such nutrients, originating from detritus that had beenb buried in the past, it new to the water, and is an input. Except for catastrophic overturn of the sediment they are available only during anoxia in the deep water.

The sediments are potentially a vast source of nutrients (Bray et al., 1973). Because anoxia in the deep water occurs during the time of year of greatest potential productivity, the nutrients originating in the sediment may have an important role in the nutrient dynamics and ecology of an estuary. It is important, therefore, to be able to distinguish the sources of the nutrients in the deep anoxic water of an estuary in summer.

The purpose of this paper is to describe how observed vertical nutrient and salinity profiles in the anoxic deep water of a stratified estuary can be used to determine the rate of nutrient production in the water relative to the rate of nutrient transports through the water. The method is based on the solution of partial differential equations for transport of salt and of a non-conservative nutrient, transformed as suggested by Officer and Lynch (1981). It is used to show that, while only about half of the nutrients observed in the deep anoxic water at a site in the lower Potomac River estuary was produced in the water at the site, the remainder was transported to the site with salt from higher salinity water farther down the estuary, little or none having entered the water from the bottom.

Application of the method to observations made in the main stem of the Chesapeake Bay will be described in another paper.

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Y. Mazurek for technical assistance; and the crew of the R/V Warfield and R/V Aquarius, without whose aid none of the observations could have been made.

Methods

The sampling station was located in the Potomac estuary 12 miles upstream from its confluence with the Chesapeake Bay at Lat. 38° 06′ 32″ and Long. 76° 32′ 44″;. The mean depth was 12 m. A submersible pump was lowered every hour between 1100 on 23 July 1979 and 1100 on 27 July 1979 from the R/V Warfield and raised at 1 m intervals from within 1 m of the bottom to the surface. Temperature and conductivity were measured every meter. Salinities were determined from these measurements. Water samples, taken at 2 m intervals, were analyzed for dissolved oxygen (Carpenter, 1965), dissolved orthophosphate (Murphy and Riley, 1962) and ammonia (Solorzano, 1969), after filtration through Whatman GF/F filters. All analyses for dissolved orthophosphate were performed on-board ship within 6 hr of sample collection. A smaller number of filtered samples were frozen and transferred to the R/V Aquarius where analyses for ammonia were performed within 24 hr.

Although data were collected for 96 consecutive hours, only data for the 43 hr period between 1200 on 23 July and 0700 on 25 July 1979 are considered here. Intrusion of a new water mass introduced sharp discontinuities into the observed patterns after 0700 on 25 July 1979.

Results

Observed concentrations (μM) of dissolved orthophosphate and ammonia increased with increasing depth during each traverse through the water column. The concentrations varied with time, however, at each depth. Similarly, salinity (°/oo) increased with increasing depth but varied with time at each depth. The variation with time coincided, roughly, with variation in tidal motion, although amplitudes were different at different depths and times.

The nutrient concentrations at any salinity, however, did not vary with time. This is illustrated in Fig. 1, in which measured dissolved orthophosphate concentrations have been plotted against salinity for the 43 hr period, and in Fig. 2 in which measured ammonia concentrations have been plotted against salinity for a 13 hr period. The scatter among the points shown is apparently random; there are no discernable time-dependent patterns.

Dissolved orthophosphate concentrations were uniform at about 0.2 μM for salnity less than 10.2 °/oo and increased curvilinearly between 10.2 °/oo and 14.0 °/oo. Similarly ammonia concentrations were uniform at about 1 μm for salinities less than 10.2 °/oo and increased curvilinearly between 10.2 °/oo and 14.0 °/oo. In both cases the curvature is convex upward.

Observed dissolved oxygen concentrations (ml at STP 1^{-1}) have been plotted against salinity in Fig. 3. The concentrations were independent of time at each salinity. Except near 8 °/oo the profile is linear. The least squares regression line shown corresponds to the equation

$$DO = -2.12S + 21.6 \tag{1}$$

where DO represents dissolved oxygen concentrations smaller than 5 ml 1^{-1} and S represents salinity.

The salinity at which the dissolved oxygen concentration reached zero was 10.2 °/oo (+0.5 °/oo, -0.1 °/oo, within a 95 percent confidence interval). The data points of Fig. 1 and 2 within the salinity range 10.2 °/oo and 14.0 °/oo represent, therefore, observations made in the anoxic water at the sampling station.

Theory

The partial differential equations for the transport of salt and of a non-conservative substance, like a nutrient, are

$$\frac{\partial S}{\partial t} + v_x \frac{\partial S}{\partial x} + v_y \frac{\partial S}{\partial y} + v_z \frac{\partial S}{\partial z} = \frac{\partial}{\partial x} (K_x \frac{\partial S}{\partial x}) + \frac{\partial S}{\partial y} (K_y \frac{\partial}{\partial y}) + \frac{\partial}{\partial z} (K_z \frac{\partial S}{\partial z}) \tag{2}$$

$$\frac{\partial C}{\partial t} + v_x \frac{\partial C}{\partial x} + v_y + v_y \frac{\partial C}{\partial y} + v_z \frac{\partial C}{\partial z} = \frac{\partial}{\partial x} (K_x \frac{\partial C}{\partial x}) + \frac{\partial}{\partial y} (K_y \frac{\partial C}{\partial y}) + \frac{\partial}{\partial z} (K_z \frac{\partial C}{\partial z}) + P \tag{3}$$

in which S is the salinity (°/oo); C is the nutrient concentration (μM); t is the time (s); x, y, z are the position coordinates (m); v_x, v_y, v_z are the flow velocities in the x, y, z directions (ms^{-1}); K_x, K_y, K_z are the turbulent (eddy) diffusion coefficients in the x, y, z directions (m^2 sec^{-1}); and P is the rate of nutrient production by anaerobic mineralization in the water ($\mu M\ s^{-1}$). The flow velocities, the turbulent diffusion coefficients and the production rate are, in general, functions of x, y, z, and t.

As suggested by Officer and Lynch (1981), application of the chain rules for differentiation

$$\frac{\partial C}{\partial x,y,z} = \frac{\partial C}{\partial S} \; \frac{\partial S}{\partial x,y,z} \qquad (4a,\ b,\ c)$$

$$\frac{\partial^2}{\partial x,y,z} \; \left(\frac{\partial C}{\partial S}\right) = \frac{\partial^2 C}{\partial S^2} \; \frac{\partial S}{\partial x,y,z} \qquad (5a,\ b,\ c)$$

and

$$\left(\frac{\partial C}{\partial t}\right)_{x,y,z} = \left(\frac{\partial C}{\partial t}\right)_s + \left(\frac{\partial C}{\partial S}\right)_t \; \left(\frac{\partial S}{\partial t}\right)_{x,y,z} \qquad (6)$$

transforms Eq. 2 and 3 into

$$\left(\frac{\partial C}{\partial t}\right) = \hat{K} \; \frac{\partial^2 C}{\partial S^2} + P \qquad (7)$$

in which

$$\hat{K} \equiv K_x \; \left(\frac{\partial S}{\partial x}\right)^2 + K_y \; \left(\frac{\partial S}{\partial y}\right)^2 + K_z \; \left(\frac{\partial S}{\partial z}\right)^2 \qquad (8)$$

It should be noted that in Eq. 3 the time derivative $(\frac{\partial C}{\partial t})$, is evaluated at constant x, y, and z, whereas in Eq. 8 it is evaluated at constant S.

When the nutrient concentration at any salinity is independent of time, the time derivative drops out of Eq. 7, which becomes

$$\hat{K} \frac{d^2C}{dS^2} + P = 0 \qquad (9)$$

With the assumption that the ratio $P/\hat{K}$ is independent of salinity Eq. (9) can be integrated between $S = S_b$ where $C = C_b$ and $S = S_a < S_b$ where $C = C_a < C_b$ to give

$$\frac{C-C_a}{C_b-C_a} = \frac{S-S_a}{S_b-S_a} \left(1+\Phi \frac{S_b-S}{S_b-S_a}\right) \qquad (10)$$

in which Φ is a dimensionless parameter given by

$$\Phi = \frac{P}{(C_b-C_a)} \quad {\frac{2\hat{K}}{(S_b-S_a)^2}}^{-1} \qquad (11)$$

The form of Φ reveals it to be a ratio of a characteristic specific nutrient production rate to a characteristic specific diffusion rate in the water in the salinity range $S_b \geq S \geq S_a$. It is the equivalent of the second Damkohler number (Alves and Boucher, 1959) used by chemical engineers in descriptions of chemical reactions combined with diffusion in fluid systems.

When $\Phi = 0$, in the absence of production, the concentration/salinity profile is linear. When $0 < \Phi < 1$, the profile is convex upward. When $\Phi \geq 1$, there is a concentration maximum at

$$S_{max} = \frac{(\Phi+1)S_b + (\Phi-1)S_a}{2\Phi} \tag{12}$$

If it is assumed that the net vertical advective velocity is very small, the net upward flux of nutrient is given by

$$j = -K_z \frac{dC}{dz} = -K_z \frac{dC}{dS} \frac{dS}{dz} \tag{13}$$

As the bottom is approached the salt flux, $K_z (\frac{dS}{dz})$, must approach zero (Officer, 1976; p. 193). Thus, the observation that C is a function of S infers that there is no flux of nutrient from the sediment.

At the depth where $S = S_a$ the nutrient flux, j_a is given by

$$j_a = -K_{za}(1+\Phi \frac{C_b - C_a}{S_b - S_a}) \left.\frac{dS}{dz}\right|_a \tag{14}$$

This consists of two parts. One is the flux of nutrient transported passively with salt in the salinity range $S_b \leq S \leq S_a$

$$j_{a_1} = -K_{za} \frac{C_b - C_a}{S_b - S_a} \left.\frac{dS}{dz}\right|_a \tag{15}$$

The other is the flux caused by production

$$j_{a_2} = -K_{za} \, \Phi \, \frac{C_b - C_a}{S_b - S_a} \Big| \frac{dS}{dz} \quad a \qquad (16)$$

which is independent of nutrient concentration.

The ratio, R, of the flux caused by production to the total flux is

$$R_a = \frac{\Phi}{(1+\Phi)} \qquad (17)$$

This is the fraction of the nutrient being transported upward in the anoxic water at a depth corresponding to $S = S_a$ that was produced in the water in the salinity range $S_b \leq S \leq S_a$. If S_a is taken to be the salinity at which the dissolved oxygen concentration reached zero, then R represents the fraction of nutrient produced in the anoxic deep water.

Application to Data

The applicability of Eq. 10 to the data pairs within the salinity range 10.2 $^o/oo$ to 14.0 $^o/oo$ shown in Fig. 1 and 2 was tested by means of non-linear parameter estimation. The method used was the LSQ routine of the Time Series Processor, Version 217 (Hall, 1975) available at The Johns Hopkins University Computing Center. The end points were taken at S_a = 10.2 $^o/oo$ where C_a = 0.2 μM for soluble orthophosphate and 1 μM for ammonia and S_b = 14.0 $^o/oo$. Because neither soluble orthophosphate nor ammonia concentration could be specified unequivocally at S_b = 14.0 $^o/oo$, C_b was allowed to be a parameter to be estimated along with Φ in each determination.

The estimated values of the parameters Φ and C_b for the 124 data pairs shown in Fig. 1 and the 26 data pairs shown in Fig. 2 are listed in Table 1. Estimates of the standard deviations, σ_Φ and σ_{C_b}, and the coefficients of determination, R^2, are also listed. The estimates of Φ are statistically indistinguishable from each other. The curves corresponding to Eq. 10 for these estimates of the parameters have been drawn in Fig. 1 and 2.

The value of R is 0.53 for dissolved orthophosphate and 0.51 for ammonia. Approximately half of these nutrients diffusing upward were produced by mineralization within the anoxic water at the sampling station.

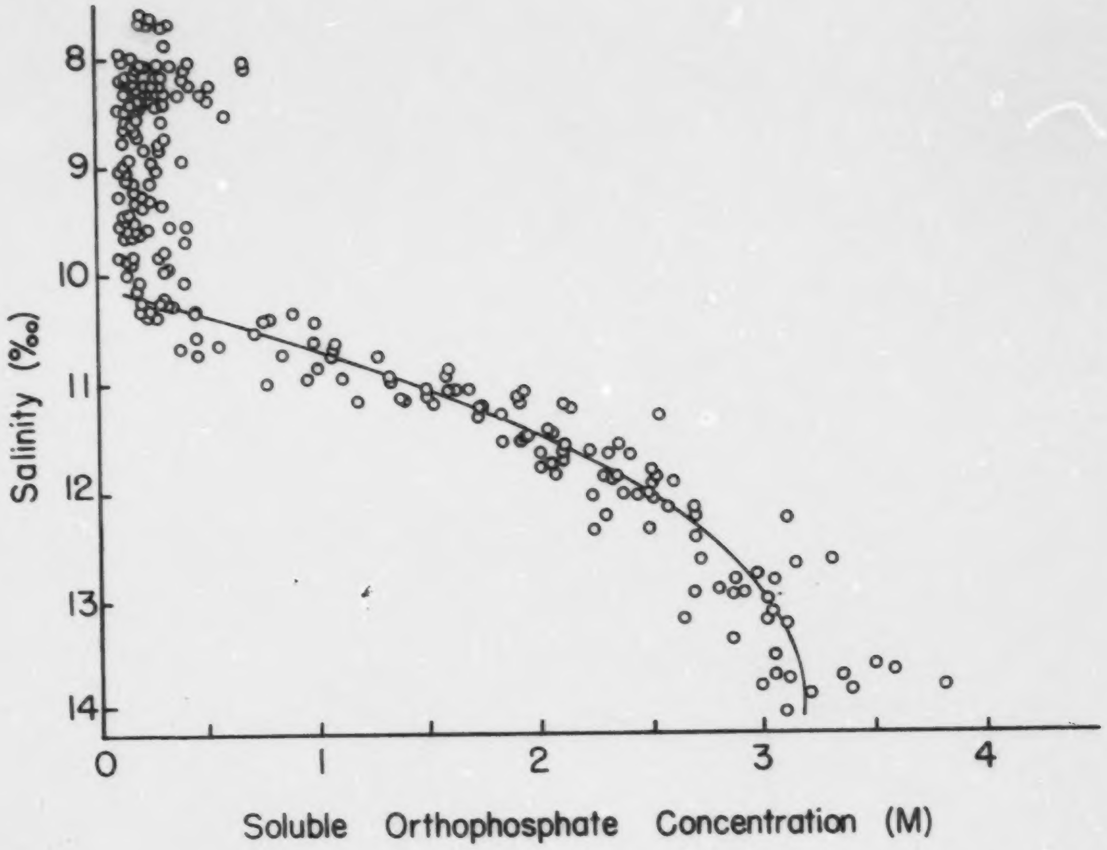

Figure 1. Soluble orthophosphate concentration as a function of salinity. The curve drawn between S_a = 10.2, C_b = 0.2, and S_b = 14.0, C_b = 3.18 corresponds to Equation 10 with Phi = 1.13.

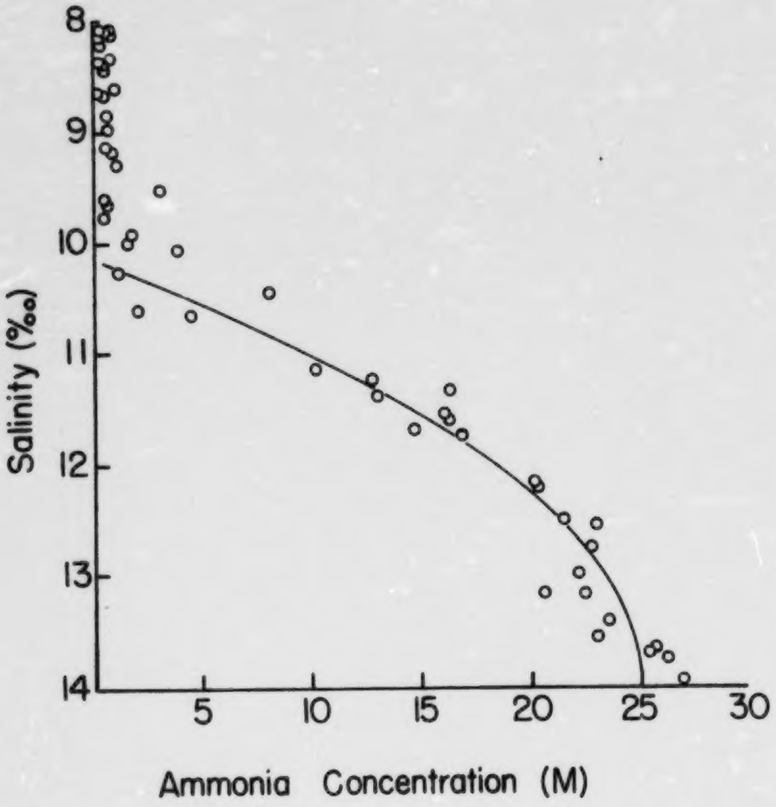

Figure 2. Ammonia concentration as a function of salinity. The curve drawn between $S_a = 10.2$, $C_a = 1.0$ and $S_b = 14.0$, $C_b = 25.03$ corresponds to Equation 10 with Phi = 1.03.

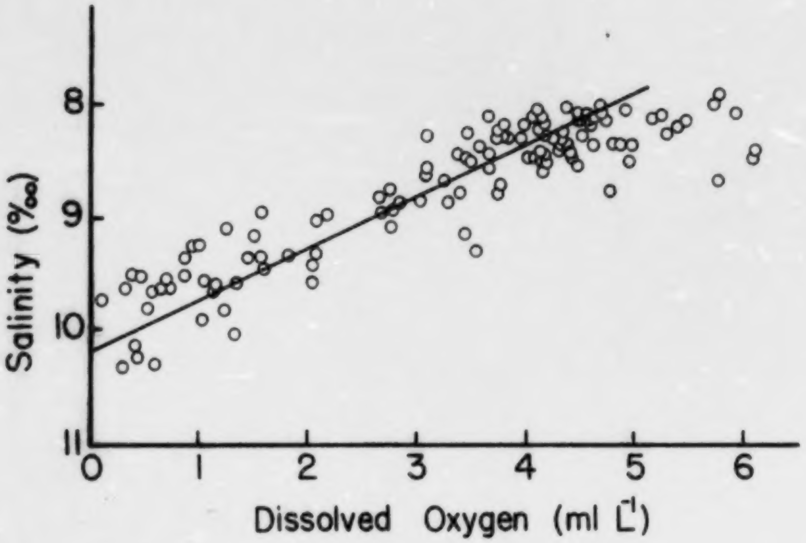

Figure 3. Dissolved oxygen as a function of salinity. The curve drawn corresponds to Equation 1.

TABLE 1

Estimates of the Parameters C_b and Φ Resulting from Application of Eq. 10 to the Data Pairs Illustrated in Fig. 1 and 2.

Data Pairs	C_b	σ_{C_b}	Φ	σ_{Φ}	R^2
Soluble orthophosphate/ Salinity	3.18 µM	0.10	1.13	0.16	0.89
Ammonia/Salinity	25.03 µM	0.88	1.03	0.16	0.94

Discussion

The assumption that the nutrient concentration/salinity profiles were independent of time made in the derivation of Eq. 10 was valid for the observations made in the lower Potomac River estuary during the 43 hr sampling period between 1200 on 23 July 1979 to 0700 on 25 July 1979. Afterward, intrusion of a new water mass introduced rapid changes in the profiles. Evidently the integrity of the water mass in the vicinity of the sampling station had been maintained, despite tidal oscillation, at least during the 43 hr period. This time period is consistent with the 2.5 day time scale of ''classical estuarine circualtion'' patterns observed by Elliot and Wang (1978) to occur in the lower Potomac River estuary about 43% of the time.

That statistically significant values of Φ could be estimated for both the dissolved orthophosphate and ammonia/salinity profiles suggests that the assumption that $P/\hat{K}$ made in the derivation of Eq. 10 was a valid one. Moreover, because S and P and $\hat{K}$ are all functions of the space coordinates and time that cannot be inverted, there is no reason to expect that $P/\hat{K}$ is a function of salinity.

Because of the flux of nutrient from the sediment into the overlying water must be zero, if C is a function of S, the ability of Eq. 10 to fit the data allows the inference that the flux from the sediment was indiscernably small relative to the nutrient transported from higher salinity water and produced in the water at the sampling station. It is not possible to determine the magnitude of the flux nor of the production rate from the data. The data allow only the comparison to be made, through the estimation of ϕ, of the production rate in the water to the transport rate through the water.

That half of the nutrients transported into the water at the sampling station must have originated in the main channel of the river, in water deeper than that at the sampling station, or more likely, in the deep central trough of the Chesapeake Bay. There ocean water entering over the relatively shallow sill that extends approximately up to the mouth of the Rappahannock River descends to depths of 20 m or more and slowly moves up the Bay. Salinity changes very little with height above the bottom in the trough; relatively little mixing occurs, especially in summer. Nutrients can accumulate owing to mineralization of detritus, leading to anoxia, and possibly also by diffusion from the sediment. As the nutrient bearing water in the trough flows landward, it mixes into the water abnove, providing the nutrients observed being transported through lower salinity water farther up the estuary.

While these results do not unequivocally rule out a nutrient source in the anoxic sediment, they do show that, at least at the time and place the observations were made, the input from the sediment was indistinguishably small relative to the nutrients produced in and transported through the water overlying the sediment. This is consistent with the estimate made by Bray et al (1973) that the anoxic sediment of the Chesapeake Bay estuarine system can supply but 5% of the observed nutrients in the Bay.

The ratio of the ammonia concentration to that of dissolved orthophosphate was 7-8 in the anoxic water. The ratio was about 5 in the oxygenated water, where S > 10.2 °/oo. If most of the nutrients were released from phytoplankton detritus, the ratio would be expected to be closer to 16.

Nixon (1979, Table 2) observed that in Narragansett Bay the ratio was 7.6 for ''recycled'' nutrients, the bulk of which entered the water from the sediments. He attributed the discrepancy to denitrification. He reasoned that ammonia, released during aerobic oxidation of detritus on the bottom, was oxidized to nitrate ion. This was then reduced to nitrogen gas by anaerobic denitrifying bacteria when bioturbation and burrowing microfauna mixed the upper layers of the sediment into a reducing environment deeper in the sediment.

Boynton et al (1980) obtained a ratio of 6.3 in the nutrient flux out of the oxidized upper layers of the sediment of the Patuxent River estuary, a tributary of the Chesapeake Bay. They also observed that 41 percent of the bound nitrogen settling to the bottom with detritus remained in particulate form and was buried. Of the remaining nitrogen 34 percent was returned to the water and 24 percent could not be accounted for. Boynton et al. reasoned that both burial and denitrification contributed to the low ammonia/dissolved orthophosphate ratio they observed.

Denitrification is not possible in the anoxic deep water nor in the reduced sediment surface, since ammonia must first be oxidied to nitrate ion in order for denitrification to take place. Evidently a smaller fraction of bound amino-nitrogen than of bound phosphate was released by anaerobic mineralization of detritus as it settled through the anoxic water. A larger fraction of bound amino-nitrogen than of bound phosphate must then have been buried in the sediment.

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CHAPTER VIII

Sediment Exchanges of Nutrients and Oxygen in Chesapeake Bay

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ABSTRACT

Sediment nutrient fluxes and oxygen consumption (SOD) were measured *in situ* along with vertical profiles of sediment and water-column nutrients, and plankton photosynthesis (P_a) and respiration (R_n) at 8 locations along the salinity gradient of Chesapeake Bay during 2 distinctive seasons. Strong spatial and temporal patterns emerged. Highest rates of sediment NH_4^+ flux, SOD, P_a and R_n occured in summar in the mid-salinity (12-17^o/oo) region, suggesting a strong coupling of these processes. Sediment fluxes of dissolved inorganic phosphate were always low, possibly due to high redox conditions. Nitrate (NO_3^-) fluxes were directly proportional to NO_3^- concentrations in overlying waters, and seasonal shifts in sediment O:N flux ratios suggest that denitrification occurred in spring but not summer. Significant relationships were observed between indices of the quality and quantity of sedimenting materials and NH_4^+ flux (less so for SOD), and C:N:P composition of surficial sediments was also consistent with the magnitude and direction of seidment fluxes. Accumulation rates of particulate nitrogen in sediments were similar to those of NH_4^+ flux in the low salinity region but less than 4% of NH_4^+ flux in other areas, suggesting that burial may be a significant term in nitrogen budgets in some estuarine zones. SOD was found to be important in oxygen dynamics at our stations (13-68% of

total respiration), and sediment fluxes of NH_4^+ were capable of suppling 9-40% of phytoplankton demand, being more important during the summer period of highest productivity.

INTRODUCTION

Estuarine sediments represent the cumulative history of biogeochemical processing of materials transported in the mixing of rivers with the sea. Production and degradation of organic matter provide the primary energy sources for early diagnesis which involves a sequence of oxidation-reduction reactions distributed vertically in the sediment column. Considerable attention has been focused on the remineralization of organics in estuarine and coastal marine sediments, and the number of direct measurements of sediment nutrient regeneration and oxygen consumption is increasing (e.g. Zeitzschel 1980; Nixon 1981). Within a given estuary several studies have considered benthic remineratlization along the salinity gradient by examining pore-water profiles (Matisoff et al. 1975; Martens and Goldhaber 1978) and by direfct measurements of nutrient fluxes (Elderfield et al. 1981; Callender 1982). At present, the relationship between these sediment processes and the mixing of estuarine waters above (e.g. Sholkovitz 1976; Liss 1976) remains unclear, and there are few accepted generalities relative to spatial and temporal patterns.

Understanding the factors which regulate remineralization processes in aquatic sediment has also been a matter of concern and a few broad correlative trends have emerged. For example, the importance of annual plankton production and water column length have been demonstrated for some lacustrine, coastal and estuarine sediments (Hargrave 1973; Smith et al. 1978; Suess 1980; Zeitzschel 1980; Nixon 1981; Fisher et al. 1982). Various investigators have reported significant correlations of sediment oxygen demand (SOD) with temperature and sediment characteristic (Hargrave 1969; Pamatmat 1977). Others have demonstrated the influence of macroinvertebrate activity on nutrient fluxes across the sediment-water interface (Aller 1978; Henriksen et al. 1981). However, such direct relationships do not always hold, being complicated by non-linear interactions among factors (Pamatmat 1977; Kemp and Boynton 1981).

The importance of sediment remineralization processes can be viewed, in part, in terms of their influence on pelagic dynamics. Limnologists have traditionally considered the role of sediments on the loss of dissolved oxygen (O_2) from hypolimnetic waters during periods of summer stagnation (Hutchinson 1941; 1957; Edmondson 1966; Hargrave 1973; Lasenby 1975). Recently, a similar perspective has been applied for estuarine systems, including San Francisco Bay (Peterson 1979) and Chesapeake Bay (Taft et al. 1980; Kemp and Boynton 1980). Few of these studies, and none for estuaries, have directly assessed the overall importance of SOD for O_2 dynamics in an entire ecosystem.

Nutrients regenerated by estuarine sediment processes also serve to supply a portion of the demand for phytoplankton productivity. The significance of this sediment source seems to vary among estuarine and coastal systems, ranging from <10% of the demand to >100% (Nixon 1981;

Harrison 1981). There are presently insufficient direct simultaneous measurements of both benthic and pelagic nutrient regeneration to develop broad generaliations as to their relative importance, and the matter remains controversial (Rowe et al. 1975; Carpenter and McCarthy 1978). While measurements of Boynton et al. (1980) have shown that benthic recycling supplied most of the phytoplankton nutrient requirement in a Chesapeake Bay tributary, and Carpenter and McCarthy (1978) have indirectly estimated the role of zooplankton excretion for the main portion of this estuary, no direct observations have been reported to address this question.

In the present paper we provide direct *in situ* measurements of ammonium (NH_4^+), nitrate (NO_3^-) and dissolved inorganic phosphate (DIP) regneration and O_2 consumption for sediments in the spring and summer at eight stations distributed along Chesapeake Bay's salinity gradient. Using statistical correlative analysis and examining spatial patterns we infer possible factors regulating sediment remineralization of organic matter. Finally, we estimate the potential influence of sediment diaganic processes on nutrient losses, O_2 budgets and phytoplankton production for a large estuarine area.

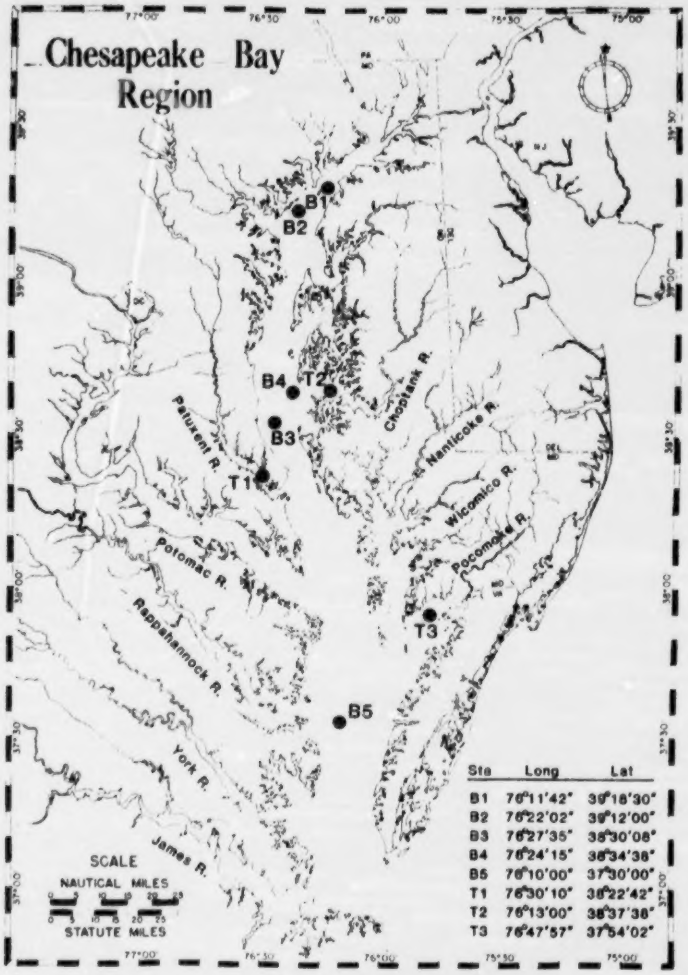
THE STUDY AREA

This study was conducted in Chesapeake Bay which is a coastal plain estuary characterized by moderate stratification (Pritchard, 1967). The estuary is about 300 km in length and from 8 to 40 km in width (Fig. 1). Approximately 50% of the bay area is less than 6m in depth while about 35% is greater than 9m (Pritchard 1952). Our stations were located in 3 contiguous regions positioned along the main axis of the bay including the brackish upper bay (km 0-40), the mesohaline mid-may area (km 40-190) and the saline lower bay (km 190-300). Characteristics distinguishing these areas include salinity, depth, circulation, degree of stratification, turbidity, ambient nutrient concentrations and degree of seasonal anoxia, all of which have beenb previously described (Whaley et al. 1966; Pritchard 1967; Seitz 1971; Taft et al. 1980). Sampling sites were chosen to be representative of the average depth and sediment type within each zone. Cruise dates (28 July-1 August 1980 and 4-8 May 1981) were selected to coincide with periods of maximum algal production and high freshwater discharge, respectively. Additional stations were also established near the mouths of three tributaries where water depth was considerably less than in the mid and lower Bay.

MATERIALS AND METHODS

In-Situ Chamber Measurements

Replicate measurements(3-4) of sediment oxygen demand (SOD) and nutrient flux were made *in situ* at each station using opaque chambers constructed from molded acrylic or welded aluminum. Each chamber was



Sta	Long	Lat
B1	76°11'42"	39°18'30"
B2	76°22'02"	39°12'00"
B3	76°27'35"	38°30'08"
B4	76°24'15"	38°34'38"
B5	76°10'00"	37°30'00"
T1	76°30'10"	38°22'42"
T2	76°13'00"	38°37'38"
T3	76°47'57"	37°54'02"

Figure 1. Station locations in Chesapeake Bay visited in August, 1980 and May, 1981.

cylindrical in shape with a height of 18 cm and a circular base of 62 cm diameter. The chamber volume was 38 ℓ, covering a surface area of 0.3 m^2. A 10 cm wide horizontal flange was attached to the edge of the chamber to rest on the bottom. A submersible pump and O_2 probe were attached to the inside of each chamber and connected to shipboard by cables. These pumps discharged recirculated water under each chamber through a manifold and were capable of maintaining mean current speeds within the chamber of 10-15 cm s^{-1}, typical of those encountered in near-bottom waters of the study area. A detailed description of the chamber is given in Boynton et al. (1981).

Prior to a measurement, chambers were filled with water to remove all air and slowly lowered to the bottom. Placement on the bottom was accomplsihed with the assistance of SCUBA divers to ensure that the chambers were securely placed and to note penetration into the bottom. After waiting 15 min for equilibration, the pumps were started. Temperature and O_2 readings were taken at 10 min time intervals over a period lasting 1-3 h. Incubation durations were such that O_2 concentrations never decreased by more than 2 mg l^{-1} or to less than 2.0 mg l^{-1}. Water samples from within the chambers were obtained 4-5 times through each incubation period for analysis of NO_3^-, NH_4^+ and DIP. Nutrient and O_2 fluxes were estimated by calculating the mean rate of change in concentration over the incubatiuon period and then converting the volumetric rate to a flux using the volume: area ratio of the chamber. Concentration changes were generally linear over sampling periods. Nutrient and O_2 concentration changes in stirred, 20 ℓ dark bottles incubated at ambient wter temperature were used as an estimate of net fluxes in the water overlying sediments, and chamber fluxes were corrected accordingly to obtain net sediment flux.

Sediment Profiles

Vertical profiles of NH_4^+ and DIP in interstitial waters and particulate carbon (PC), nitrogen (PN) and phosphorus (PP) were also measured for sediments at each stration. Sediment cores (65 cm in depth) were obtained using a gravity corer fitted with a 6.75 cm diamter plastic liner. After the corer was recovered, the liner was removed and vertically inserted into a glove box which was purged by nitrogen gas. Sediments were hydraulically extruded in 1 cm intervals and pore water was extracted by pressure filtration (Reeburgh 1967). Pore waters were placed in containers and immediately frozen. Analysis of particulate fractions was conducted on sub-samples of the solid sediment phase. Sediment bulk densities were also determined at 1 cm (top 10 cm) and 5 cm (11 to 30 cm) intervals following the method of Gardner 1965).

Water-Column Photosynthesis, Respiration and Concentrations

Estimates of plankton photosynthesis (P_a) and respiration (R_n) were obtained using the O_2 light-dark bottle method. Triplicate light bottles were filled from and suspended *in situ* at depths approximating 90, 60, 30 and 10% of surface isolation, and replicate (3) dark bottles were filled from the upper and lower portions of the water column and incubated *in situ* at ambient temperatures. All incubations were conducted for 3-6h periods. Volumetric rates of P_a and R_n were converted to areal basis by linear interpolation between depths to the compensation level for P_a and to the bottom for R_n. At each station and date water samples were obtained from several depths for NO_3^-, NH_4^+, DIP, PC, PN, PP, seston, temperature (T), salinity (S), O_2 and attenuation of photosynthetically active radiation (PAR). Nutrient samples were processed and frozen for subsequent analysis.

Chemical and Physical Analyses

Analytical methods were as follows: NO_3^-, NH_4^+ and DIP were analyzed using the automated method of EPA (1979); total dissolved phosphate (TDP, filtered) and total phosphate (TP, unfiltered) analyses used the disgestion and neutralization procedure of D'Elia et al. (1977) followed by DIP analysis (EPA 1979); sediment PP concentrations were obtained by acid digestion of dry sediment (Aspila et al. 1976); and PC and PN samples were analyzed suing a Model 240B Perkin Elmer Elemental Analyer. Methods of Strickland and Parsons (1968) were employed for seston determinations. Oxygen concentrations were determined using an Orbisphere Model 2607 oxygens system which was calibrated prior to each use. A Beckman Induction salinometer was used to monitor T and S, while PAR attenuation coefficients were estimated using a Li-Cor submersible quantum sensor.

RESULTS AND DISCUSSION

Temporal and Spatial Patterns

Dissolved and Particulate Concentrations

In general, the concentrations of dissolved and particulate forms of nitrogen and phosphorus (Table 1) were comparable to those previously reported for this estuary (Whaley et al. 1966; Taylor and Grant 1977;

Table 1. Physical and chemical characteristics of the water column during August 1980 and May 1981 cruises. Double entries represent average values for the euphotic (top value) and sub-euphotic zones.

Location	Z_t (m)	T (°C)	S (‰)	Z_e (m)	O_2 (mgℓ$^{-1}$)	Seston (mgℓ$^{-1}$)	NO_3^- (µg-atoms ℓ$^{-1}$)	NH_4^+ (µg-atoms ℓ$^{-1}$)	DIP (µg-atoms ℓ$^{-1}$)	PC (µg-atoms ℓ$^{-1}$)	PN (µg-atoms ℓ$^{-1}$)	PP (µg-atoms ℓ$^{-1}$)
							AUGUST 1980					
Upper Bay												
B 1	4.0 -	28.8 28.2	4.6 4.8	1.6 -	6.7 5.9	- 48.7	21.10 20.30	4.28 5.90	0.65 0.65	- 106.0	- 12.10	- 1.17
B 2	4.6 -	27.0 27.2	6.6 6.8	1.9 -	6.3 5.7	- 30.5	11.50 7.60	6.40 3.20	1.00 0.59	- 82.3	- 13.50	- 1.05
Mid-Bay												
B 3	11.2 -	27.1 26.4	13.7 13.9	4.1 -	6.9 2.6	- 38.3	1.50 0.58	2.90 10.80	1.30 0.36	- 56.6	- 9.61	- -
B 4	13.2 -	26.8 26.8	13.6 13.6	4.6 -	6.7 5.9	- 29.1	1.50 0.37	2.90 0.80	1.30 0.14	- 65.8	- 12.50	- 0.34
Lower Bay												
B 5	11.2 -	28.0 24.5	17.7 24.0	7.4 -	7.4 3.9	- 57.5	- 0.68	- 5.40	- 0.66	- 41.6	- 6.94	- 0.52
Tributaries												
T 1	7.1 -	27.3 27.0	12.5 12.9	2.3 -	4.8 2.7	- 47.2	1.50 2.10	3.10 11.10	1.50 0.57	- 151.9	- 30.30	- 1.56
T 2	6.6 -	29.5 29.0	13.0 13.1	3.3 -	8.9 6.1	37.6 36.0	0.70 0.84	1.70 4.60	0.11 0.15	- 58.1	- 12.10	0.77 1.68
T 3	5.2 -	28.2 28.3	16.5 16.5	2.6 -	6.5 6.0	- 114.7	- 0.60	- 2.30	- 0.13	- 125.2	- 23.94	- 0.54
							MAY 1981					
Upper Bay												
B 1	4.0 -	13.9 14.0	2.1 2.1	2.0 -	8.95 8.80	31.0 48.0	40.50 40.70	0.40 4.60	0.20 0.20	133.3 141.5	13.70 14.20	1.70 1.50
B 2	4.6 -	15.1 14.4	6.7 7.2	2.3 -	10.35 8.10	42.0 61.0	26.50 28.10	4.50 8.40	0.10 0.10	248.0 258.0	35.40 33.20	2.21 2.50
Mid-Bay												
B 3	11.2 -	13.2 12.7	13.9 16.7	6.7 -	10.20 8.20	15.3 64.7	19.40 9.90	1.30 3.10	0.10 0.10	67.1 153.0	8.50 18.80	0.10 0.70
B 4	13.2 -	14.4 12.7	14.8 17.7	6.4 -	10.40 7.10	12.9 17.9	14.00 5.30	1.70 3.00	0.10 0.10	88.1 155.6	10.80 21.20	0.50 0.70
Lower Bay												
B 5	11.2 -	14.2 14.1	21.0 23.3	7.5 -	9.72 9.30	39.7 53.2	0.20 0.40	0.90 0.80	0.10 0.10	217.0 216.0	12.10 17.00	0.53 0.88
Tributaries												
T 1	7.1 -	12.0 9.9	15.8 17.0	3.4 -	12.20 12.40	45.3 39.0	0.23 3.00	0.50 3.90	0.11 0.18	280.0 153.0	23.50 22.70	0.37 0.79
T 2	6.6 -	15.6 15.2	15.4 15.4	9.7 -	8.50 9.20	12.2 22.0	10.80 10.50	0.70 1.20	0.09 0.10	30.0 65.0	4.20 6.70	0.00 0.37
T 3	5.2 -	14.9 15.0	19.2 20.1	7.0 -	7.85 8.50	37.3 43.0	0.19 0.20	0.60 0.60	0.11 0.10	69.0 107.0	7.80 10.80	0.35 0.68

*Abbreviations in column headings are: Z_t = total depth; T = temperature; S = salinity; Z_e = depth to 1% light; O_2 = dissolved oxygen; NO_3^- = nitrate; NH_4^+ = ammonium; DIP = dissolved inorganic phosphate; PC = particulate carbon; PN = particulate nitrogen; PP = particulate phosphorus.

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Heinle et al. 1980). Spatially, NO_3^- decreased rapdily down-estuary during both cruises NH_4^+ was generally higher in August but lacked longitudinal gradients, and DIP exhibited little pattern during the spring although higher DIP concentrations were evident in mid-bay during August. Particulate phosphorus decreased seaward during both cruises but PC and PN did not exhibit strong longitudinal patterns.

In general, bottom water concentraitons of NH_4^+ were higher than those in surface waters, while no obvious vertical trend was apparent for DIP (Table 1), suggesting that the relative magnitude of sediment regeneration of NH_4^+ was greater than for DIP. Vertical profiles of T, S and O_2 indicated a conspicuous absence of the srongly developed pycnocline which previously has been documented for the estuarine channel (e.g. Seitz 1971). For example, vertical different in T and S at the two deepest stations in the mid-bay region (B3, B4) were 0.3C and 0.2°/oo respectively, in August. In contrast, Seitz (1971) reported that normal surface to bottom T and S differences were on the order of 1-3C and 5-9 °/oo in this region of the Bay during these seaons. In addition, we found O_2 in bottom waters ranging from 35-79% of saturation at mid-bay stations during August, a period when O_2 has been previously observed at bottom waters ranging from 35-79% of saturation at mid-bay stations during August, a period when O_2 has been previously observed at 0-20% of saturation (Taft et al. 1980). Whether or not such vertically well-mixed water columns are a more common occurrence for Chesapeake Bay than previously indicated remains unclear, as does the mechanism by which this

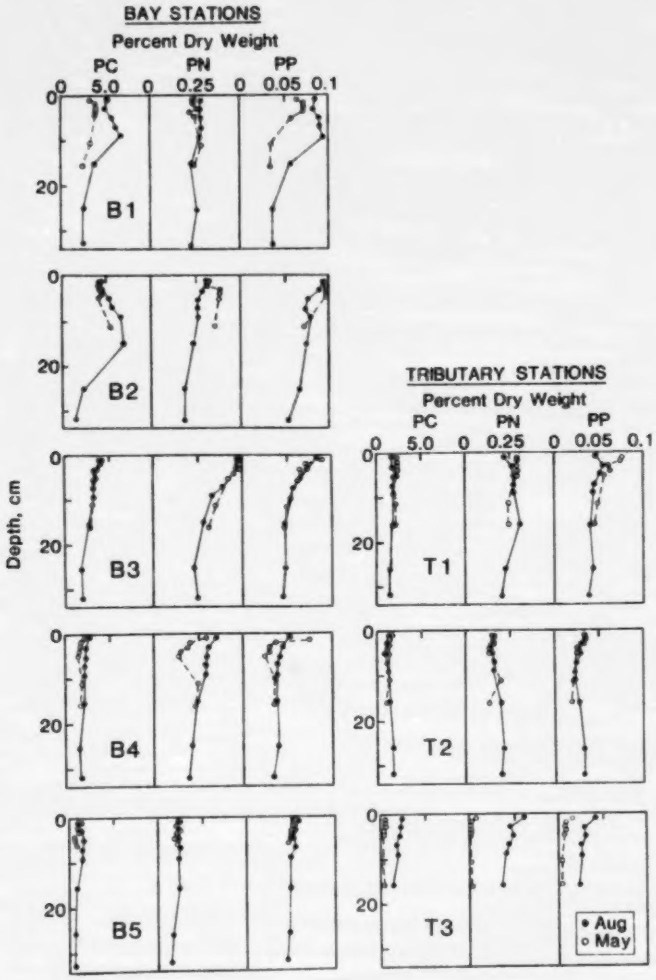

Figure 2. Vertical distribution of particulate carbon, nitrogen and phosphorus (PC, PN, and PP) in sediments at 8 locations in Chesapeake Bay in August, 1980 and May, 1981.

mixing is effected.

Sediment Profiles

Concentrations of PC (Fig. 2) ranged from about 1-7% of dry sediment weight, generally decreased with sediment depth and declined steadily from upper bay to lower bay, being significantly correlated with salinity ($r=0.79$). A notable exception to this vertical trend was observed at the two upper-bay stations where maximum PC values occurred 9-15 cm below the surface. This enhancement may have resulted from intense deposition of particulate materials in the upper bay during the passage of tropical storm Agnes (June, 1972), and the depth of this PC maximum is consistent with the event date and deposition rates (Hirschberg and Schubel (1979). Concentrations of PN ranged between 0.1 and 0.6% in surface sediments and were considerably reduced in the lower bay (Stas. B5, T3). As with PC, values of PN tended to either remain constant or decrease with depth. Particulate phosphate values ranged between 0.01 and 0.11% in surface sediments and exhibited longitudinal and vertical patterns similar to those of PC and PN. At Sta. T3 (and to a lesser extent at Stas. B2 and B4), there were considerable differences in PC, PN and PP observed between cruises. Presumably, these differences mainly represent spatial variability rather than seasonal changes in sediment composition (Matisoff et al. 1975).

While there were significant differences in particulate composition of sediments observed among stations, there was a remarkable consistency in vertical particulate C:N:P ratios at individual stations, except in the upper bay. For example, the C:N:P ratio was 132:15:1 at 1 cm and

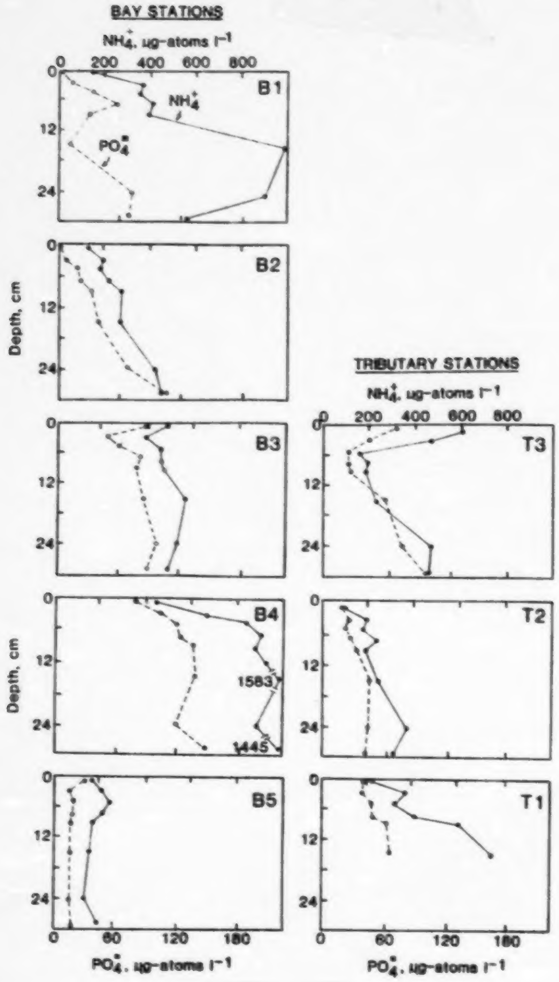

Figure 3. Vertical distribution of NH_4^+ and dissolved inorganic phosphorus (DIP) in sediment pore-waters for August, 1980 at 8 locations in Chesapeake Bay.

123:13:1 at 30 cm at Sta. B4. It appears that over a wide spectrum of estuarine conditions and long time period, diagenic processes modify PC, PN and PP concentrations in a fashion maintaining near constant proportions of these materials.

Pore-water concentrations of NH_4^+ and DIP ranged from about 100-1600 µg-atoms ℓ^{-1} and 1 - 150 µg-atoms ℓ^{-1}, respectively (Fig. 3), and were similar to those previously reported for these locations and depths (Matisoff et al. 1975). With few exceptions, concentrations increased with depth and there was a general pattern of increasing NH_4^+ and DIP concentrations from upper bay to mid-bay stations followed by a sharp decline at the most saline stations. Similar salinity related patterns of pore-water nutrient concentrations have been reported in other estuaries (Martens and Goldharber 1978; Callender 1982), and it has been suggested that such patterns result from the different types of bacterial decomposition which occur along salinity gradients as well as the nature of deposited organic material. The distinctly lower concentrations we observed at the high salinity site possibly reflect lower deposition rates of organic material and more complete remineralization near the sediment surface of labile planktonic debris (Martens and Goldharber 1978). In general, as with sediment particulates, the N:P ratios in pore-waters were remarkably constant with depth although some deviations occurred which will be discussed later.

Plankton Photosynthesis and Respiration

Phytoplankton production (P_a) ranged from 1.48 - $16.76 gO_2m^{-2}d^{-1}$ in August and from 0.83 - $6.82 gO_2m^{-2}d^{-1}$ in May (Table 2 and Fig. 4).

Our estimates of P_a were quite similar to those previously reported for similar times of the year in this estuary (Flemer 1970; Taft et al. 1980; Kemp and Boynton 1980). Maximum values occurred most frequently in the mid-salinity regions of the Bay. Of the 21 P_a values in excess of $5gO_2m^{-1}d^{-1}$, 17 were recoreded between salinities of 13 - 17°/oo. This pattern, which is also apparent in the data of Flemer (1970), may be attributable to the relative balance between high nutrient concentrations and low turbidity in this zone of the estuary (Table 1). In contrast, lower salinity waters may tend to be limited by light availability while nutrient depletion may limit phytoplankton in the more saline reaches (Kemp et al. 1982). Patterns for R_n were similar to those for P_a, with the ratio P_a:R_n generally greater than 1.0 in spring and < in summer (Table 2; Fig. 4).

Sediment Fluxes

Out SOD values (Table 2 and Fig. 4) were quite comparable to those reported for other productive estuarine and coastal environments (Smith 1973; Nixon et al. 176; Boynton et al 1980) and to previous measurements made in the vicinity of Sta. B3 (Kemp and Boynton 1981). There were no significant differences (one-way ANOVA, Student-Newman-Keuls test; $P<0.05$) between rates measured in August and May at the same stations, none among stations in August and only a few significant differences among stations in May [T3> (B1, B2, B3, B4, T1, T2))>B5]. In contrast to the seasonal and temporal differences observed in water column P_a and R_n, SOD rates were quite invariant.

Table 2. Apparent Production and night respiration of plankton and sediment fluxes of oxygen, ammonium, nitrate and phosphate at eight locations in Chesapeake Bay. Negative sign indicates flux from water to sediments; ND indicates non-detectable net flux.

Station	Season	Water Column Fluxes		Sediment Fluxes			
		Plankton Production (Pa) ($gO_2m^{-1}d^{-2}$)	Plankton Respiration (Rn) ($gO_2m^{-2}d^{-1}$)	Sediment Oxygen Demand (SOD) ($gO_2m^{-2}d^{-1}$)	NH_4^+ (μg-atoms $m^{-2}h^{-1}$)	NO_3^- (μg-atoms $m^{-2}h^{-1}$)	DIP (μg-atoms $m^{-2}h^{-1}$)
B1	Aug	3.20 ± 0.22	6.80 ± 0.24	1.45 ± 0.35	176.6 ± 14.1	288.4 ± 41.6	-4.2 ± 0.7
	May	2.05 ± 0.11	1.73 ± 0.32	1.94 ± 0.06	47.5 ± 10.8	-117.6 ± 10.7	3.2 ± 0.1
B2	Aug	1.53 ± 0.05	9.84 ± 1.62	1.68 ± 0.07	102.0 ± 17.3	85.2 ± 2.2	2.3 ± 0.2
	May	6.68	3.86	2.18 ± 0.06	60.4 ± 20.6	-63.4	ND
B3	Aug	16.44 ± 0.30	14.60 ± 0.83	2.86 ± 0.98	669.7 ± 189.1	-10.0 ± 0.8	40.2 ± 16.8
	May	2.81 ± 0.41	1.52 ± 0.18	1.90 ± 0.16	60.7 ± 14.6	-53.8 ± 3.6	ND
B4	Aug	6.99 ± 0.95	17.42 ± 0.91	3.28 ± 1.35	454.5 ± 150.0	ND	ND
	May	2.13 ± 0.29	2.14 ± 0.64	1.70 ± 0.42	110.2 ± 3.3	-33.3 ± 0.1	ND
B5	Aug	12.52 ± 0.42	1.84 ± 0.80	1.45 ± 0.21	261.9 ± 72.9	ND	9.9 ± 8.6
	May	2.60	4.82	1.54 ± 0.08	35.6	ND	6.0
T1	Aug	11.13 ± 1.17	9.84 ± 0.48	1.96 ± 0.31	432.8 ± 24.8	41.2	21.2 ± 8.5
	May	6.46 ± 0.31	5.83 ± 1.01	2.03 ± 0.14	32.4 ± 12.6	ND	ND
T2	Aug	7.13 ± 0.30	12.29 ± 1.67	1.85 ± 0.36	411.4 ± 8.1	17.2 ± 1.7	16.4 ± 3.7
	May	1.66 ± 0.14	0.83 ± 0.15	1.76 ± 0.07	72.3 ± 0.1	ND	ND
T3	Aug	11.54 ± 0.44	1.63 ± 0.30	2.72 ± 0.02	430.6 ± 231.4	39.6	3.2 ± 5.5
	May	0.93 ± 0.13	0.92 ± 0.24	3.15 ± 0.26	81.9 ± 2.6	ND	ND

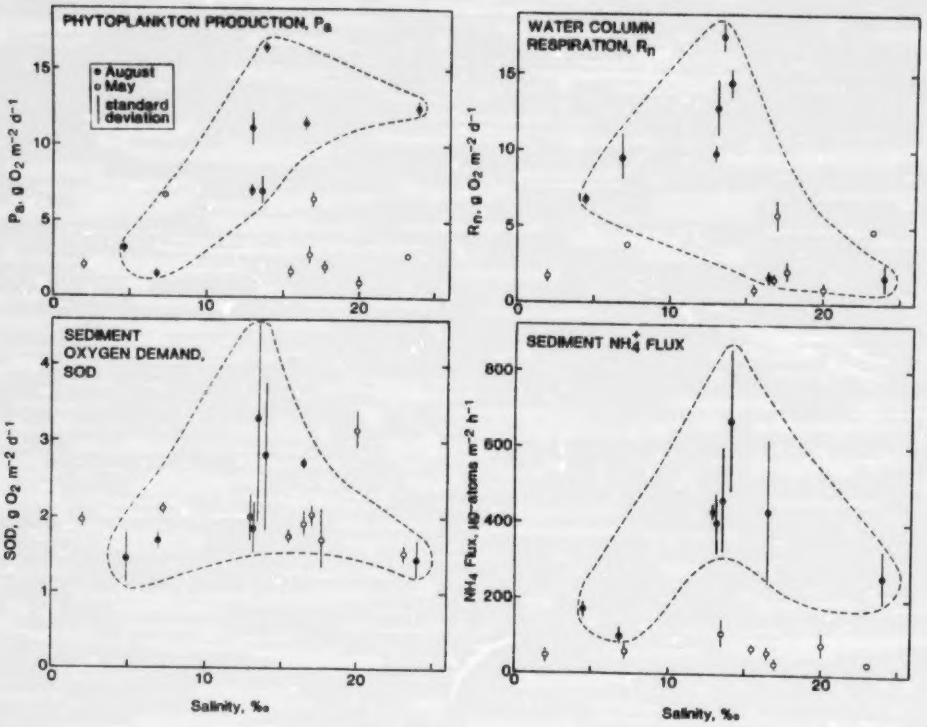

Figure 4. Average rates (± standard deviation) of phytoplankton photosynthesis (P_a), water column respiration (R_n), sediment oxygen demand (SOD), and NH_4^+ flux as a function of salinity. Envelopes are drawn around August rates to emphasize spatial patterns.

Ammonium fluxes across the sediment-water interface ranged from 91.3 to 821 µg-atoms N $m^{-2}h^{-1}$ during the summer and contrasted sharply with rates observed in May (35.6 - 99.8 µg-atoms N $m^{-2}h^{-1}$) which were lower and spatially less variable (Table 2 and Fig. 4). These rates are well within the range of those summarized by Zeitzschel (1980) and Nixon (1981). There were no statistically significant differences among stations in May; however, two statistically separate groups were evident in August, with higher value in tributaries and mid-Bay areas than in the upper and lower bay, a pattern similar to that observed for P_a. In two other studies where spatial patterns of NH_4^+ and DIP flux were examined, some trends were evident. For example, Elderfield et al. (1981) found highest fluxes at a low salinity station, while the data of Callendar and Hammond (1982) suggest little pattern for NH_4^+ flux but highest DIP fluxes occurred at salinities similar to those where we found maximum benthic remineraliation.

Nitrate was observed both leaving and entering sediments during the study (Table 2 and Fig. 4). Large net fluxes were observed only in the upper bay (B1 and B2) where NO_3^- was released from sediments in August and consumed by sediments in May. During the May cruise a more consistent pattern was evident where fluxes were directed into sediments, decreasing in magnitude in a down-bay direction. Nitrate fluxes were inversely correlated with salinity (r^2=0.76) and directly proportional to NO_3^- concentrations in the overlying water (r^2=0.83). Spring rates of NO_3^- uptake were comparable both to those reported previously for the Patuxent estuary (Boynton et al. 1980; 1982) and to direct measurements of sediment denitrification of $^{15}NO_3^-$ from overlying water in Japanese coastal sediments during the same season (Koike and Hattori 1978a; 1978b; Nishio et al. 1982). Rates of NO_3^- production for Stas. B1 and B2 were similar with those observed in Narragansett Bay (Nixon et al. 1976).

Sediment fluxes of DIP were erratic in late summer, near zero in the spring and overall quite low compared to those observed in a Chesapeake Bay tributary (Boynton et al. 1980) and other estuaries (Nixon 1981). Furthermore, these DIP fluxes were <10% of those expected on the basis of observed NH_4^+ regeneration and N:P composition of deposited organic matter (Table 2 and Fig. 4). A plausible explanation for these low DIP fluxes would involve the formation of insoluble mixed iron-manganese phosphate complexes and/or sorption to oxyhydroxides, both of which occur under oxic conditions (Bray et al. 1973; Khalid et al. 1978; Krom and Berner 1980). Taft and Taylor (1976a) inferred from O_2 and DIP relationships in the water column and DIP release from sediments under anoxic conditions was an important source in the deep waters of Chesapeake Bay. Klump and Martens (1981) found tht _in situ_ DIP fluxes at Cape Lookout Bight were often less than predicted by pore-water concentration gradients when O_2 was present in overlying waters, suggesting that DIP removal occurred at the sediment-water interface. Vertical profiles of Eh revealed that sediment pore-water were highly reduced at all of our stations during the August cruise (Jenkins 1981), and this coupled with oxygenated bottom waters suggests that the same mechanisms may have been operative here as in Cape Lookout Bight. Since oxidized conditions were observed in May in the sediments and O_2 levels were near saturation in the water, it is probable that DIP immobilization occurred even deeper in the sediment profile and resulted in almost no DIP flux at any of our stations.

Sediment Composition and Regenerative Fluxes

There are numerous factors which have been suggested to influence sediment remineralization of organic matter (Hargrave 1973, 1980; Pamatmat 1977; Zeitzschel 1980; Nixon 1981), and among the most important of these are the quantity and quality of material deposited from overlying water. While we do not have specific quantitative measures of deposition rates for our 8 stations, it is clear that the upper bay (Stas. B1 and B2) experiences the highest sedimentation rates (Schubel and Hirschberg 1977). However, regenerative fluxes of NH_4^+ and SOD were relatively low at these stations (Table 2), and presumably fine-grain inorganics dominate deposition in this region of the estuary (Postma 1967; Schubel 1968). Phytoplankton debris probably reprsents the most important source of high quality organic substrate for benthic remineralization (Nixon et al. 1980, Kemp et al. 1982), and various elemental composition ratios (C:N:P) can be used as an index of planktonic source (Redfield 1934). In the following we consider benthic regenerative fluxes in relation to organic production of plankton, and to these elemental ratios in seston and in surficial sediments (0-1 cm).

The ratio C:N is coastal and estuarine particulates provides a reasonable index of organic matter source, with ratios of about 6-8 typical of plankton detritus (Muller 1977; Smetacek et al. 1978; Walsh 1981). Patterns of seston and sediment C:N along the estuarine salinity gradient of our stations are evident (Fig. 5 a,b) where seston C:N ratios range between 4-13 at all stations and both dates. There is some indication of enrichment in N compred to phytoplankton composition in August particularly in the mid-bay stations where NH_4^+ regeneration rates, SOD and P_a were greatest (Table 2). All measurements of C:N in sediments were above 7.0 and exceeded 20 in the upper bay, implying a preferential regeneration of N relative to C or possibly representing the higher C:N ratios typical of terrestrial debris (Muller 1977) in the region closet to the riverine source. This pattern of a more pronounced terrestrial influence in the upper estuary is consistent with that observed by Brinson et al. (1982) using $\delta^{13}C$ for the Neuse and Pamlico estuaries and by Brush and DeFries (1981) using pollen analysis in the Potomac. AT the brackish stations there was also a strong modification of C:N from seston to sediment (comparing Fig. 5 a and b), with Δ(C:N) averaging about 7 in May and 13 in August at Stas. B1 and B2, again suggesting more rapid recycling of N compared to C. Similar diagenic decreases in C:N have been reported by others for coastal sediments (Stevens et al. 1967; Hargrave and Taguchi 1978).

For seston, the ratio N:P may be taken to indicate relative nutrient limitation for phytoplankton growth (Rhee 1978), while N:P may also represent the relative decomposition rates of detrital N versus P in seston or sediments. Seston N:P along the Chesapeake Bay axis exhibited a wide range (from 5-44), with values at or below phytoplankton composition occurring primarily in upper and lower bay (Fig. 5c). In the tributaries and mid-bay region where P_a, SOD and NH_4^+ flux were maximal,

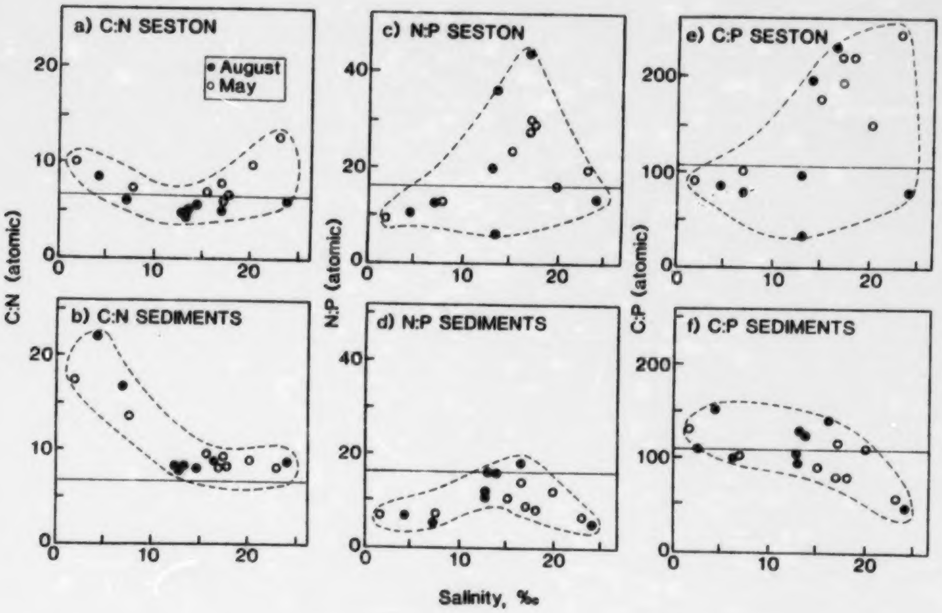

Figure 5. Composition ratios of particulate material along the salinity gradient in Chesapeake Bay for seston C:N (a), N:P (c), C:P (e) and surficial sediment (0-1 cm) C:N (b), N:P (d), C:P (f). Envelopes are drawn around data to emphasize spatial patterns. Horizontal lines indicate expected Redfield composition ratios.

N:P exceeded 20 for 7 or 8 observatios. These relatively high ratios may be the result of P-limited algal growth, and others have reported evidence of P limitation periodically in Chesapeake Bay (Taft et al. 1975; Taft and Taylor 1976b). This interpretation is consistent with our observations of high NH_4^+ to DIP ratios measured in the water column at Stas. B3, B4, T1, T2 (Table 1), and of large N but negligible P regeneration for these sediments (Table 2). In fact, a significant correlation was found between mean N:P in seston and NH_4^+ regeneration in sediments (r=0.77). Similarly, sediment values of N:P were mostly below phytoplankton composition (Fig. 5d), indicating a relatively slow release of DIP, except in the mid-bay region which is apparently prone to summer anoxia with rapid relese of DIP (Taft and Taylor 1976).

By comparing C:P ratios in particulate matter, further qualitative evidence is obtained regarding organic sources and regeneration rates. Seston C:P exhibited a pattern similar to N:P, further emphasizing the likelihood of P-limited phytoplankton during our cruises (Fig. 5e). In the sediments, C:P did not vary greatly from Redfield proportions, but exceeded 130 at Sta. B1, while C:P < 100 in the lower may have resulted from P bound in Fe-Mn oxy-hydroxide complexes in these aerated sediments (Fig. 5f). Moreover, the radical shift of particulate C:P from seston to sediments in mid to lower estuary implies marked differential diagenesis of P relative to C. Thus, the patterns apparent in the C:N:P composition of suspended and recently deposited particulates were consistent with _in situ_ measurements of NH_4^+, DIP, and O_2 flux across the sediment-water interface.

Observed rates of SOD and NH_4^+ flux exhibited some significant correlations with several factors Table 3). Temperature showed strong effect on NH_4^+ regeneration, but not for SOD. Other investigators have reported similar relations for nutrient regeneration (e.g. Nixon 1981), and we previously found temperature to be a surprisingly poor predictor of SOD (Kemp and Boynton 1981). Lacking direct measurements of planktonic deposition, we used P_a as an index of short-term plankton detritial production. In some studies there was considerable temporal lag between peak P_a and plankton sedimentation (Smetacek et al. 1978; Smetacek 1980; Jewson et al. 1981; Bodungen et al. 1981) and between deposition and decomposition (Jewson et al. 1981; Graf et al. 1982) and between deposition and decomposition (Jewson et al. 1981; Graf et al. 1982). Nevertheless, we found a highly significant relationship between NH_4^+ regeneration and P_a ($r=0.82$), indicating a strong benthic-pelagic coupling, probably in both directions. The weak correlation for SOD versus P_a may be the consequence of less direct dependence of SOD on plankton deposition, since the former represents the oxidation of reduced substances (H_2S, CH_4) generated deep in the sediments as well as recently sedimented organics.

The relative amount of N in particulate organic material is a key factor influencing its susceptibility to remineralization (Toth and Lerman 1977; Hargrave 1980; Hanson 1982). The C:N ratio of seston significantly correlated with NH_4^+ regeneration in the sediments ($r=0.67$), but the relation was again weak for SOD. For superficial

Table 3. Correlation coefficients (r) for sediment oxygen demand (SOD) and sediment NH_4^+ regeneration relative to indices of plankton detritus and its production. Subscripts s and w refer to surficial sediments and water column, respectively. Values in parenthesis were calculated using August data only. Abbreviations are: P_a =apparent production; PC = particulate carbon; PN = particulate nitrogen; SOD = sediment oxygen demand.

Remineralization Rate	Temperature	P_a	PN_s (%)	$(PC:PN)_w$	$(PC:PN)_s$
SOD	0.14	0.28	0.25 (0.68**)	-0.21	-0.36 (0.53*)
NH_4^+ Flux	0.77**	0.82**	0.31 (0.55*)	-0.67**	-0.38 (0.73**)

* Significant at $p < 0.15$.
** Significant at $p < 0.05$.

sediments both C:N and %N exhibited significant relationships to both SOD and NH_4^+ in August, but the correlations were lost with the inclusion of spring data. Apparently, deposition rates of low C:N detritus were sufficiently low in August to compensate for the high ratios in relict material (Walsh et al. 1981). The conclusion of this analysis is that rates of organic matter remineralization are, indeed, highly dependent on the nature and rate at which organic substrate is supplied.

Stochiometric Flux Ratios, Denitrification and Burial

The aerobic decomposition of organic matter derived from phytoplankton results in the generation of about 16 atoms of N and 1 atom of P for every 276 oxygen atoms consumed (Redfield 1934; Alvarez-Borrego et al. 1975). Nixon and his colleagues (Nixon et al. 1976; 1980; Nixon 1981) have discussed the possible implications of O:N and N:P ratios in remineralization fluxes across the sediment-water interface. Actual fluxes for Narragansett Bay sediments exhibited considerable departure from stochiometric ratios predicted by the Redfield model, with values of O:N and N:P tending to be anamolously high and low, respectively (Nixon et al. 1976; 1980). Similar patterns have been reported for other coastal sediments (Nixon 1981); however, some systems seem to follow the Redfield prediction more closely (e.g. Fisher et al. 1982). The proportionately low release of N (mostly as NH_4^+) from sediments to overlying water has been attributed to high rates of denitrification for Narragansett Bay (Nixon 1981; Seitzinger et al. 1981).

We observed distinctly different patterns of O:N flux ratios between spring and summer cruises (Fig. 6). For August fluxes, mean O:N for

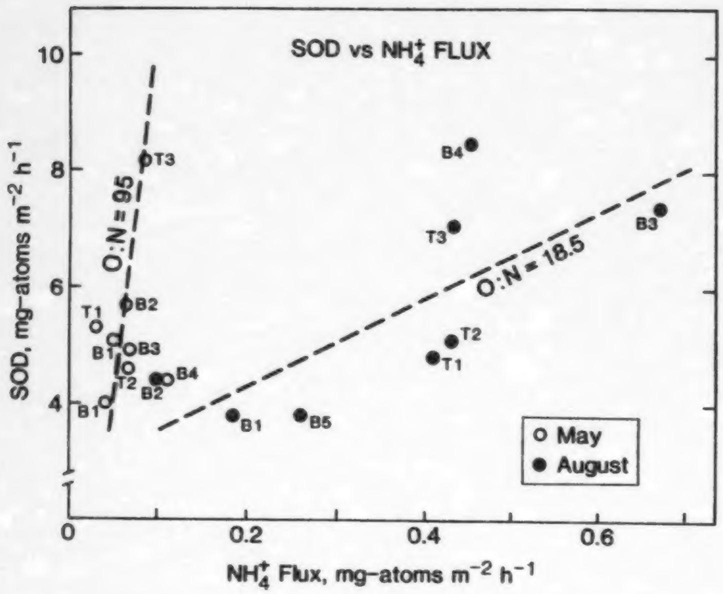

Figure 6. Relationships between sediment NH$_4^+$ flux and SOD in August (solid circles) and May (open circles) at 8 station locations in Chesapeake Bay. The slopes of lines through August and May data points are equal to respective mean O:N ratios.

eight stations was consistent with the Redfield model being 18.5 considerating only NH_4^+ regeneration and 13.8 including NO_3^- evolution as well. A significant correlation was obtained here for O_2 consumption versus NH_4^+ recycling ($r^2=0.55$). Conversely, the mean O:N flux ratios for May mesurements were 182 and 95 with and without consideration of NO_3^- uptake. Obviously, in the spring processes other than simple aerobic decomposition of organic matter were operative. We have no direct measurements of denitrification in these sediments. However, Jenkins and Kemp (1982) have reported strong seasonal trends for the coupled nitrification-deitrification reaction in a tributary of Chesapeake Bay (Patuxent Estuary). Rates of about 160-200 µg-atoms $m^{-2}h^{-1}$ were measured in April compared to 0.6-1.0 µg-atoms $m^{-2}h^{-1}$ in August. The spring rates are similar to the eman NH_4^+ regeneration rates in the Patuxent during that period (Boynton et al. 1980; 1982), and thus are capable of accounting for a doubling of the O:N flux ratio. The conspicuous lack of DIP regeneration from these sediments rendered N:P flux ratios (30-40) in excess of Redfield proportions for both dates.

Fluxes of NO_3^- across the sediment-water interface exhibited a strong seasonality which is consistent with the invocation of denitrification to explain anamolous O:N ratios (Fig. 6). Generally, and particularly in the upper bay stations, NO_3^- was consumed by sediments in May and evoked from sediments in August. Based on these NO_3^- fluxes on O:N remineraliation ratios, it appears that denitrification in May, coupled to sediment nitrification, occurred throughout the estuary with denitrification of water column NO_3^- also extant at upper bay stations.

Despite the considerable nitrification (NO_3^- evolution from sediments) occurring in the upper bay in August, denitrification was evidently negligible. This is in contrast to the findings of Jenkins and Kemp (1982) for the Patuxent Estuary, where no nitrifiction could be detected in August.

Another possible explanation for the discrepancy between observed stochiometric ratios for benthic fluxes and phytoplankton elemental proportions would be differential burial of N relative to C or P. The rate of C, N or P burial (F_i) can be estimated from the following relation

$$F_i=C_i(\rho)\omega \qquad (1)$$

where C_i is the % composition of particulate material buried for species i, ρ is the dry bulk density of solids buried and ω is rate of sediment accretion. Various estimates of ω are available for upper and middle Chesapeake Bay (Biggs 1970; Schubel and Hirschberg 1977; Goldberg et al. 1978; Hirschberg and Schubel 1979), and from these we took values of 9.0 and 1.2 mm y^{-1} for Stas. B1 and B4, respectively. Applying these deposition rates to our measurements of C_n (1.25% N for both stations, Fig. 2) and ω (0.42 and 0.46 g cm^{-3} for B1 and B4) at 20 cm sediment depth, we calculate long-term means of 96 and 8 µg-atoms m^{-2} h^{-1} for burial of N at Stas. B1 and B4, respectively.

At the upper bay station this rate is almost identicl to the mean of NH_4^+ fluxes measured on the two sampling dates, while it is less than 4% of the mean NH_4^+ regeneration for Sta. B4 (Table 2). Hence, burial is potentially important in the N budget of upper bay sediments, while less so for the middle bay. However, to be a significant source of the anamoly in O:N and N:P flux ratios observed for benthic regeneration of organic matter, sediment particulates must be enriched in N relative to C and depleted relative to P. In fact, we found no enrichment of N relative to C at any of our stations, while considerable depletion of N relative to P was apparent in upper bay sediments (Fig. 7). The marked bulge in particulate C:N at about 16 cm of Sta. B1 (Fig. 7a) sediment is probably a relict of the major depositional event occurring in 1972 (Schubel and Hirschberg 1977). The low particulate N:P at that station, especially in the upper 10 cm (Fig. 7b), corresponds to the high dissolved N:P ratio in pore-waters, and this relationship may represent scavenging of P by iron and manganese oxides in the upper aerated sediments (e.g. Bricker and Troup 1975; Callender 1982). At Sta. B4 the particulate ratios, C:N and N:P, are remarkably constant with depth and differ only slightly from Redfield proportions (Fig. 7d,e), indicating an absence of differential diagenic processing of these elements. The slight enrichment in particulate N:P and depletion of dissolved N:P in the upper 20 cm may represent the effects of denitrification in thse sediments. The stochiometric profiles for all mid and lower bay as well as tributary stations followed patterns similar to those for Sta. B4.

The lack of direct experimental evidence necessarily renders this analysis open to question. However, these data do suggest that an explanation for seasonally varying stochiometry of organic matter decomposition lies in annual patterns of denitrification and DIP

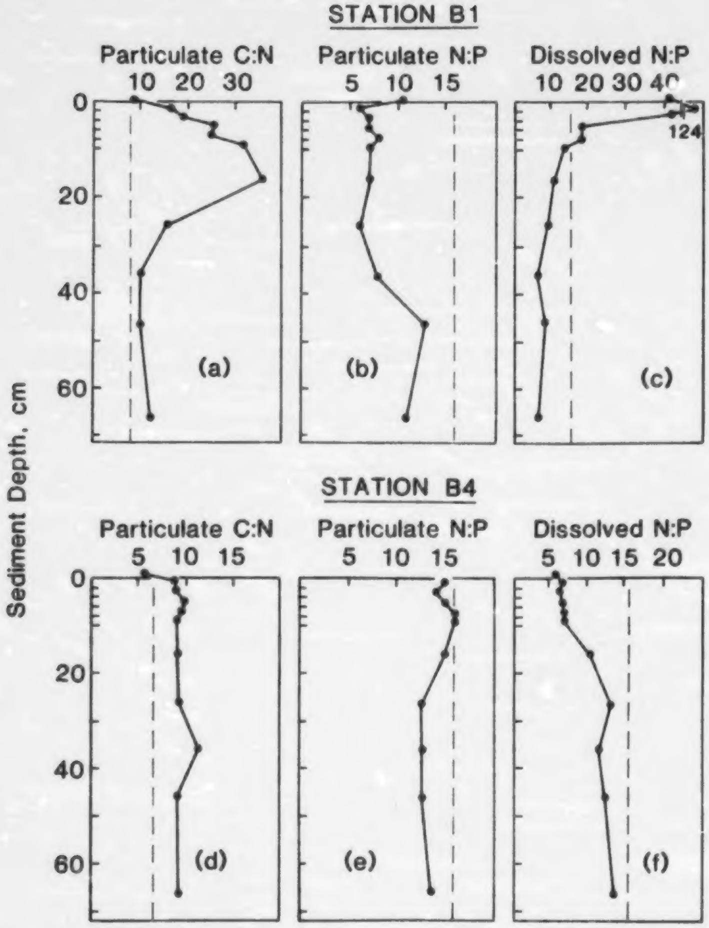

Figure 7. Vertical composition ratos (atomic) Of particulate and dissolved materials in sediments for August at staions B1 and B4.

precipitation-dissolution, both of which are in turn influenced by rates or organic matter deposition and sediment redox potential

Influence of Sediment Processes on Estuarine Waters

An important relationship by which sediments influence overlying waters is through the impact of SOD on O^2 budgets. This is of particular interest in Chesapeake Bay where anoxic conditions in bottom waters (>10 m) have been observed since the late 1930's (Newcombe and Horne 1938) and have persisted through the last decade (Taft et al. 1980). Bottom water anoxia tends to be particularly pronounced in the mid-salinity region of this estuary and is less important in the upper and lower bay. Low O^2 conditions also occur periodically in several tributaries of the Bay (Webb and D'Elia 1980).

One way to evaluate the importance of SOD on oxygen conditions is to compare this flux with total community respiration (R_t=R_n+SOD). We found that SOD ranged from 13-6% of R_t (Table 4) and displayed no clear spatial or depth-related patterns. The relative contribution of SOD to R_t was higher in May (48 + 19%) than August (25 + 18%), largely attributable to strong seasonal changes in R_n rather than SOD. These results are similar to those of Peterson (1979) who calculated that SOD represented about 50% of total community respiration in San Francisco Bay. Similarly, we fond that SOD represented 30-40% of R_t during the summer in Chesapeake Bay near St. B3 (Kemp and Boynton 1980). In contrast, Taft et al. (1980) estimated that seasonal O_2 depletion in Chesapeake Bay was primarily due to water column respiration. However, SOD was not measured directly, and the value they used ($0.5gO_2m^{-2}d^{-1}$) was lower by a factor of 3-6 than values we routinely observed. An additional method of assessing SOD as a biological component of oxygen budgets is to compare the net daily change in O_2 due to water column processes (P_a-R_n) versus SOD (Table 4). Considerable variability in net O_2 change due to plankton metabolism (-10.4 to $107 gO_2m^{-2}d^{-1}$) was apparent, but overall this ΔO_2 was close to zero at our stations. When SOD was added as a loss term, daily rates of change in O_2 became generally negative. In this view, SOD was comparable in magnitude to net changes in O_2 due to biological processes in the water column and, moreover, was large enough in 8 of 11 cases to shift daily O_2 changes from positive to negative.

A second pathway of benthic-pelagic coupling which we can evaluate involves the release of nutrients from sediments to overlying waters. We estimate the potential influence of this process by comparing phytoplankton demand for NH_4^+ to that supplied by sediment fluxes and by calculating the turnover time of the water column NH_4^+ stock based solely on sediment fluxes (Table 5). We have limited discussion to NH_4^+ because this appears to be the preferred source of nitrogen in most areas of the bay (McCarthy et al. 1975). Since we did not have direct measurements of phytoplankton nitrogen demand, we estimated this by using Redfield composition ratios and phytoplankton productivity data. Given the considerable spatial and short-term (diel) variability associated with instantaneous measurements of nutrient uptake (Kuenzler et al. 1979; Caperon et al. 1979), the ratio approach may be more appropriate for calculating longer-term average rates. Our method is also supported by the fact that C:N ratios of

Table 4. Estimates of the relative importance of SOD and plankton metabolism for O_2 dynamics during August and May at 8 stations in the Chesapeake Bay.

Station		Date	SOD Relative to Total Respiration (%)[a]	Net ΔO_2 Due to Plankton Metabolism ($gO_2m^{-2}d^{-1}$)[b]	Net ΔO_2 Due to Total Metabolism ($gO_2m^{-2}d^{-1}$)[c]
Upper Bay	B1	Aug	18	-3.6	-5.1
		May	53	0.3	-1.6
	B2	Aug	15	-8.3	-10.0
		May	36	2.8	0.6
Mid Bay	B3	Aug	16	1.8	-1.0
		May	56	1.3	-0.6
	B4	Aug	16	-10.4	-13.7
		May	44	0.0	-1.7
Lower Bay	B5	Aug	44	10.7	9.2
		May	24	-1.6	-3.1
Tributaries	T1	Aug	17	1.3	-0.7
		May	26	0.6	-1.4
	T2	Aug	13	-5.2	-7.0
		May	68	0.8	-0.9
	T3	Aug	63	9.0	7.2
		May	67	0.0	-3.1
MEAN		Aug	25 ± 18	-0.5 ± 7.9	-2.6 ± 7.9
		May	48 ± 19	+0.5 ± 1.3	-1.5 ± 1.2

[a] Calculated as sediment oxygen demand (SOD) divided by the sum of plankton respiration (R_n) plus SOD

[b] Calculated as apparent plankton production (P_a) minus R_n.

[c] Calculated as P_a minus the sum of R_n plus SOD.

suspended material largely approximated those expected of phytoplankton (Fig. 5).

We found that sediment fluxes of NH_4^+ represented from 9-40% of calculated photosynthetic demand, and averaged 27 and 15% in August and May, respectively. There was a distinct spatial pattern, with sediment remineralization most important in mid-bay (where P_a was also largest) and decreasing in both upbay and down-bay directions. In addition, there was a distinct seasonal pattern wherein the percentage of nutrient demand supplied by the sediments was higher in the summer than spring, paralleling the trend in P_a. Comparison of water column NH_4^+ stocks with phytoplankton demand also suggested a close coupling between nutrient utiliation and remineralization similar to that observed in other areas (Harrison 1978; Caperon et al. 1979; Stanley and Hobbie 1981). On average, there was about a 1-2 day supply of NH_4^+ in the water during our study (Table 5) and benthic NH_4^+ fluxes were a more important term in replenishing water column NH_4^+ during the season of highest NH_4^+ utilization.

It appears that sediment remineralization of NH_4^+ at our stations exerted a substantial influence on overall nutrient dynamics with marked spatial and temporal patterns. Nixon et al. (1976), Boynton et al (1980), Fisher et al. (1982) and others (see Zeitzschel 1980) have reached similar conclusions concerning the importance of sediment remineralization. However, the significance of this source seems to vary. Other studies indicate that water column remineralization is a dominant source and can supply up to 100% or more of phytoplankton demand (e.g. Harrison 1978; Caperon et al. 1979; Hattori et al. 1980). In some systems, including Chesapeake Bay, it appears that both are important, and it may be that total remineralization often exceeds phytoplankton demand, although it is not possible to assess this since contemporaneous measurements of both water column and sediment remineralization have not been reported for any system. A logical next step in improving our understanding of benthic-pelagic couplings would involve contemporaneous measurements of remineralization relative to total water column demand of both phytoplankton and bacteria over appropriate spatial gradients and temporal intervals.

Table 5. Estimates of the potential influence of sediment NH_4^+ fluxes on phytoplankton NH_4^+ demand and turnover of NH_4^+ stock in the water column.

Location	Date	Benthic Regeneration (mg-atoms $m^{-2}d^{-1}$)	Phytoplankton Demand*: Uptake (mg-atoms $m^{-2}d^{-1}$)	Phytoplankton Demand*: Benthic Supply (%)	Water Column Stocks: Stock (mg-atoms m^{-2})	Water Column Stocks: Benthic Turnover** (d)
Upper Bay	Aug	3.3	12.2	27	21.3	6.4
	May	1.3	6.2	21	19.2	14.9
Mid-Bay	Aug	13.5	33.9	40	52.6	3.9
	May	2.1	11.2	18	27.5	13.4
Lower-Bay	Aug	6.3	48.9	13	60.5	5.8
	May	0.9	10.2	9	9.5	12.2
Tributaries	Aug	10.2	38.9	26	28.7	2.8
	May	1.5	11.8	13	7.9	5.3
MEAN	Aug	8.3 ± 4.5	33.5 ± 15.5	27	40.8 ± 18.7	4.7 ± 1.7
	May	1.4 ± 0.5	9.9 ± 2.5	15	16.0 ± 9.1	11.5 ± 4.2

*Average rates of phytoplankton production for August and May periods were estimated using longer-term (3-6y) data bases of Flemer (1970) and Mihursky et al. (1978) for upper and mid-bay areas, respectively, in addition to data in Table 2. Rates for the lower-bay and tributaries were calculated using data in Table 2.

**Time (d) required for benthic regeneration to replace water column NH_4^+ storage.

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APPENDIX A

QUALITY ASSURANCE PLAN

CHESAPEAKE BAY NUTRIENT DYNAMICS STUDY

This plan was developed with the considerable assisance of Ms. Romona Trovata, Central Regional Laboratory, EPA, Annapolis, Maryland.

I. INTRODUCTION

The purpose of this manual is to detail procedures for sample handling and processing. It is intended to serve as the Quality Assurance Plan for the Chesapeake Bay Nutrient Study and may be incorporated into both the cooperative agreement EPA and Johns Hopkins and the sub-contracts from Johns Hopkins to other institutions.

II. SAMPLING

Samples will be collected within 100 m of current meter moorings designated by hydrography study at each depth where current velocity is measured. Samples will also be taken in the upper 1 m and 1 m from the bottom in each location. Samples will also be taken at 5 depths evenly spaced in the river mouths where current meters are not deployed in order to achieve representative coverage. Samples may be collected by pump or bottle cast. Pump systems will be flushed with two system volumes prior to sampling at each depth.

For transects 2 through 9 (Figure 1) samples will be collected twice each 24 hour period as specified by modelling group. The time of sampling is not critical but it must be recorded accurately in Eastern Standard Time. The time between successive samplings at one point should not be less than five hours. In addition to twice daily sampling, transect 1 will be sampled every 3 h for a 36 h period to be determined later depending on weather conditions.

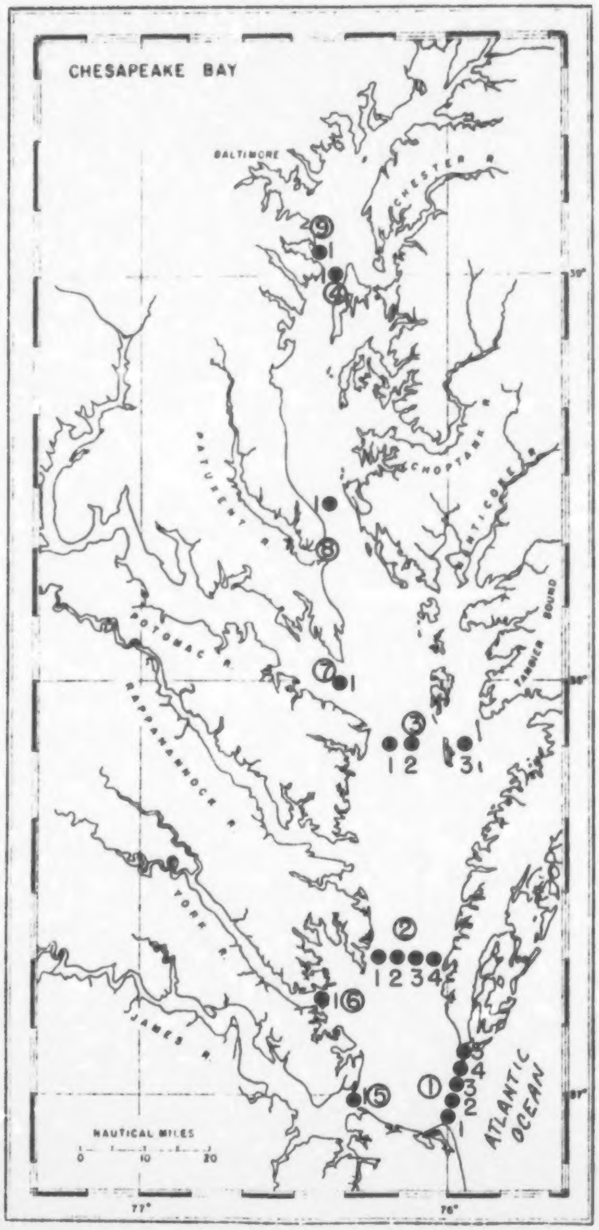

Figure 1. Map showing current meter locations and numbering for transects (within circles) and stations along each transect. Exact locations given in text.

III. FILTRATION

Samples requiring filtration will be kept out of direct sunlight and filtered within 30 minutes of collection. Glass fiber filters will be used with the suction pressure differential across the filter not exceeding 250 mm Hg (1/3 atmosphere). The specific anlytical methods give additional details.

STATION LOCATIONS

Transect #	Station #	Station Lat/Long.	Depths m
1	1	36-56-42/76-01-30	1,3,7,11,17
	2	36-57-57/76-00-38	1,3,7,11,17,19
	3	36-59-36/76-00-06	1,3,7,11
	4	37-01-42/75-59-42	1,3,6
	5	37-03-48/75-58-39	1,3,11
2	1	37-19-46/76-13-24	1,3,7,9
	2	37-19-37/76-11-09	1,3,10,13
	3	37-19-37/76-08-30	1,3,9,12
	4	37-19-12/76/05/25	1,3,10,18
	5	37-19-00/76-02-54	1,3,9
3	1	37-52-39/76-09-58	1,3,9,18,28,35
	2	37-52-45/76-06-03	1,3,8
	(TS) 3	37-53-13/75-57-10	1,3,9,17,24
Calvert Cliffs	1	38-33-30/76-26-32	1,3,9,17,20

IV. PREPARATION

Suspended Solids

Samples for suspended solids will be prepared in accordance with USEPA method 160.2 on 47 mm glass fiber filters. Care will be taken to wash the filters as indicted. At least 1 liter of sample will be filtered in the Lower Bay. For Upper Bay stations filters may clog with less than 1 liter filtered. In this instance the volume filtered will be adjusted accordingly and carefully recorded on snap lid petri dish and data sheet. Filters will be stored in plastic snap-lid petri dishes (Falcon #1006 or equivalent).

a. Chlorphyll a

Single samples for Chlorophyll a will be collected on 25 mm glass fiber fileters by filtering 25 ml as above. However, at each sampling point 1 m from the surface, triplicate Chlorophyll a samples will be collected. Filters will be folded, placed in aluminum foil to exclude light, labeled, and frozen until analysis.

b. Particulate Carbon, Nitrogen, Phosphorus

Filters for particulate carbon and nitrogen analyses need no pretreatment. Duplicate samples will be collected but not analysed until all singles are completed. Filters for particulate phosphorus analysis should be rinsed with 20 ml 6 N HCl, rinsed with 50 ml distilled water and dried prior to use.

Triplicate 100 ml samples will be filtered on 25 mm glass fiber filters (2 for CN, 1 for P). Filters will be rinsed with 20 ml filtered sea water folded in half and placed in labelled stamp envelopes (2 CN filters in one envelope, 1 P filter in a second envelope). Filters will be dried at 60°C for 24 hr and stored in a dessicator or dried and frozen. Blanks will be prepared for each station at each sampling time by passing 20 ml filtered sea water through a filter.

c. Soluble Constituents

400 ml of sample will be filtered through a 47 mm glass fiber filter which is prerinsed immediately before use with 50 ml freshly deionized water (Deeminac filter, Fisher Catalog Number 9-035-220. Deeminac filter beads should be moist and expanded to fill the cartridge when received. Dried out demineralizers should be rejected.)

The filtrate should be dispensed as follows afte rinsing storage container with at least 20 ml of filtrate. Container should not be completely filled to allow for expansion on freezing.

Number of 50 ml tubes filled	Analysis to be done	Color Code	Institution
2 plastic	SRP, NO_3, NO_2, NH_4	Yellow	HI
1 plastic	SiO_2	Pink	USGS
1 plastic	TDN, TDP	Green	CBL
1 plastic	Filtered archive	Blue	AFO
1 glass (10 ml)	DIC, DOC	Pink	VIMS

This lable system to be used is Fisher Catalog Number 11-867-50 with associated tape and labels. The labels survive freezing without coming off. The plastic tubes to be used are Fisher Catalog Number 5-538-60 with rim seal caps.

The sample identification code will be written on each tube or filter container with waterproof ink.

SAMPLE IDENTIFICATION CODE

F - 09 - 1400 - 01 - 2 - 10 - 50 - P

Filtered (F) or Whole (W) water sample

(omit for sample on filters)

Date

Time (EST)

Transect (See Map)

Station (See Map)

Depth (m)

For Filters Add

Volume Filtered (ml)

Analysis To Be Done

Carbon-Nitrogen (CN), Phosphorus (P),

Chlorophyll a (A), Suspended Solids (SS)

V. ANALYSIS

a. Methods

Samples will be analysed according to methods in Table 1. The calibration procedures in Table 2 will be followed.

For Chlorophyll a, filters will be ground in 90% acetone and extracted 12 h at 4°C. Fluorescence will be recorded before and after addition of 2 drops 1N HCl for pheophytin correction. Fluorometer calibration will be accomplished following the SCOR/UNESCO formula as given in Strickland and Parsons (1972).

For nutrient analyses performed by autoanalyzer, the methods provided by Technicon, Strickland and Parsons, or EPA are acceptable. The critical aspect of analysis is monitoring the quality of data produced in essentially real time so that problems can be identified quickly and samples re-analysed if necessary. To this end, a comprehensive protocol has been adopted from EPA, AFO to check limits on accuracy and precision during analysis. Criteria for accepting or rejecting data will be established for each instrument as indicated in Section VI. The Shewhart, CuSum, or Industrial method may be used, however, the charts should be updated as each try of samples is analysed so problems can be identified immediately.

Reference standards provided by EPA, AFO will be run each time operating standards are prepared from dry chemicals. Four standard concentrations in the sample concentration range will be run with each autoanalyzer tray and one station per tray will be duplicated. One standard spiked into sample or one operating standard will be run each 10 samples per tray as shown in the ''typical'' tray layout. All standards will be prepared at least daily from stock concentrate solutions.

TABLE 1. SAMPLE PREPARATION, STORAGE AND ANALYSIS

Parameter	Preparation and Storage	Analytical Technique	Maximum* Holding Time	Reference
Underwater Light Penetration	Analyzed Immediately	Li-Cor Quantum Sensor	not held	
Water Temp.	Analyzed Immediately	Beckman Salinometer Inter-Ocean ICTI	not held	
Salinity	Analyzed Immediately	Beckman Salinometer	not held	
Dissolved Oxygen	Analyzed Immediately	YSI and Obrisphere Oxygen meters and modified Winkler Titration		Strickland and Parsons, 1972
Suspended	Dried to Weight	Weighing		Method 160.2 USEPA, 1979
Chlorophyll a	Frozen	Fluorometric Measurement of Extract in 90% Acetone		Strickland and Parsons, 1972
Particulate Organic Carbon	Frozen or Dried at 60°C and Stored in Dessicator	Perkin-Elmer 24 OB CHN Analyzer		
Particulate Organic Nitrogen	Frozen or Dried as for Carbon	Perkin-Elmer 240B CHN Analyzer		
NO_2^-	Filtered Water, Frozen Immediately	Technicon Auto Analyzer II		Method 353.2 USEPA, 1979
NO_3^-	Filtered Water, Frozen Immediately	Technician Auto Analyzer II		Method 353.2 USEPA, 1979

NH_4^+	Filtered Water, Frozen Immediately	Technicon Auto Analyzer II	Method 350.1 USEPA, 1979
TDN	Filtered Water, Frozen Immediately	Persulfate Oxidation; Neutralization; Technicon Auto Analyzer II	D'Elia, et al., 1977 and Method 353.2 USEPA, 1979
PO_4^{-3}	Filtered Water, Frozen Immediately	Technicon Auto Analyzer II	Method 365.1 USEPA, 1979
TDP	Filtered Water, Frozen Immediately	Persulfate Oxidation; Neutralization; Technicon Auto Analyzer II	D'Elia, et al. 1972, Method 365.1 USEPA, 1979
SiO_2	Filter Water, Frozen Immediately	Technicon Auto Analyzer II	Method 370.1 USEPA, 1979
TP	Unfiltered Water Frozen Immediately	Persulfate Oxidation; Neutralization; Technicon Auto Analyzer II	D'Elia, et al., 1974, Method 365.1 USEPA, 1979
Particulate Organic Phosphorus	Frozen or Dried as for Carbon	Persulfate Oxidation; Neutralization; Technicon Auto Analyzer	D'Elia, et al., 1977 and Method 353.2 USEPA, 1979

*Samples will be analyzed within 4 h of thawing and will be held at <38°F while thawed (Strickland & Parsons, 1972).

TABLE 2. INSTRUMENT CALIBRATION AND MAINTENANCE PROCEDURE

Instrument	Calibration Procedure*	Frequency	Calibration Accuracy	Precision
Salinometers: Beckman RS5-3	(a) Temperature calibrated against precision laboratory thermometer at temperatures expected in field	Monthly		+ 0.5°C
	(b) Salinity calibrated against hydrometer at salinities expected in field and by calibration resistor	Prior to use		+ .3°/oo
	(c) Service by manufacturer	When indicated		
	(d) CBI calibration facility	9-16 June 1980		
Dissolved Oxygen Meters: YSI-54 and 57, Orbisphere 2708	(a) Calibrated against modified Winkler Method using aerated tap water	Prior to use		YSI + 0.22 ppm
	(b) Saturated air calibration	Every 3 hours		+ 0.1ppm
Analytical Balances: Mettler H-30, Sartorius 1203, Mettler 86.	(a) Zero and check level	Prior to use	100%	+ .05mg
	(b) Standard weights	Monthly		
	(c) Calibration and cleaning by manufacturer	Annualy		
CHN Analyzer: Perkin-Elmer 240-B and associated electrobalance	(a) Curves determined with standards for each parameter. Blanks also determined.	Prior to use each day and after every 6 samples		+ 10% C + 15% N
	(b) Full service by manufacturer.	When indicated		
Fluorometer: Turner Design 10, Turner 111	Preparation of standard curve of chlorophyll a in acetone extracts against spectrometer	Prior to use		+ 5%
Spectrophotometer: Gilford 240	(a) Preparation of standard curve	Prior to use daily		+ 2%
	(b) Wave length calibration with holmium filter	3 months	+ .5nm	

Bausch-Lomb Spec 100	(a) Internal calibration and standard curve	Daily	+ 2%
pH Meters: Corning 12, Orion Research 801A, 701	Calibrated using several standard buffers (pH 4, 7, 10)	Prior to use	+ .05 pH
Automated Analyzers: Technicon Auto Analyzer II Scientific Instruments CFA	(a) Curves determined with standard solutions for each parameter. (b) Operating log kept and routine maintenance performed by lab personnel (c) Full service by manufacturer	Daily prior to analyzing samples; selected standards after every 10 samples. Daily Standards will be prepared from stocks daily, as a minimum.	As determined in Quality Control Section.

*All calibrations will be noted in an instrument log book which will be available for review upon EPA request.

TYPICAL AUTOANALYZER TRAY LAYOUT

Cup #	Contents	Cup #	Contents
1	Blank, Dist H_2O	21	Sample 11
2	Blank, SW for STD	22	Sample 12
3	Standard 1 in SW	23	Sample 13
4	Standard 2 in SW	24	Sample 14
5	Standard 3 in SW	25	Sample 15
6	Standard 4 in SW	26	Sample 16
7	Blank, SW for STD	27	Sample 17
8	Sample 1	28	Sample 18
9	Sample 2	29	Sample 19
10	Sample 3	30	Sample 20
11	Sample 4	31	Blank, SW for STD
12	Sample 5	32	Std 2 in SW
13	Sample 6	33	Blank, SW for STD
14	Sample 7	34	Sample 21
15	Sample 8	35	Sample 21
16	Sample 9	36	Sample 22
17	Sample 10	37	Sample 22
18	Blank, SW for STD	38	Sample 23
19	Sample 1 and spike	39	Sample 23
20	Blank, SW for STD	40	Blank, Dist H_2O

AUTOANALYZER DATA

Date: Operator:

Samples:					I.		II.		III.		IV.		V.	
					Detn: S. Cal: Calib:		Detn: S. Cal: Calib:		Detn: S. Cal: Calib:		Detn: S. Cal: Calib:		Detn: S. Cal: Calib:	
AA Tray														
Cup #	Description													
	R/F	Time	Sta.	Dp.	Chart	Calc. Conc.	Chart	Calc. Conc.	Chart	Calc. Conc.	Chart	Calc. Conc.	Chart	Calc. Conc.
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A wide range of salinities will be encountered since samples will be collected from the head to the mouth of the bay. This leads to potential salt effect in several methods. To minimize variability due to salt effects, standards will be diluted to operating concentration in filtered sea water. For analyses in shore laboratories the appropriate salinity water for each transect (listed below) will be prepared from natural bay water or sea water, filtered through a glass fiber filter and diluted with distilled water to the appropriate salinity range. For shipboard analyses, filtered station water may be utilized. Blanks for filtered sea water will be compared to blanks in distilled water to establish the reagent blank.

Transect	Salinity for Standard
1	25°/oo
2	25°/oo
3	20°/oo
4	10°/oo
5	25°/oo
6	25°/oo
7	20°/oo
(8)	15°/oo
9	10°/oo
11*	5°/oo

*Not shown on map

b. Data Recording

Data will be recorded on log sheets in the format attached. Extra logs may be reproduced locally. Log sheets containing station data will be forwarded to Jay Taft, CBI, by 20 July. Completed log sheets for nutrient analyses including silicate will be forwarded by 1 November 1980. Completed logs for particulate C, N, P, suspended solids and DIC/DOC will be forwarded by 1 January 1980.

c. Archive Samples

One 50 ml subsample from each sampling point will be filtered and frozen for archives.

STATION LOG SHEET

Operation: ________ Station: ________ Latitude: ________ Longitude: ________

Ship: ________ Depth to Bottom: ________ Weather: ________

Date: ________ Wind: ______ ° ______ Knots

Calibration: ICTI ______ Observer: ________

Cond. Temp. Air Temperature

Hi ______ ______ Dry Bulb: ______ ° Wet Bulb: ______ °

Lo ______ ______

Time EST	Depth m	Temp.	Cond.	pH	DO	Light	EXT Chla	SiO_2	PO_4	TDP	TP	NO_2	NO_3	NH_4	TDN	Total N	PN	PC	DOC	DIC

DATA LOG FOR PARTICULATE C, N

Date Sampled: ____________ Transect: __________ Station: _________

Date Analysed: __________ Analysing Laboratory: ___________________

Time EST	Depth m	Volume Filtered ml	Zero, μV		Blank, μV		Sample, μV		PC μg at/l	PN μg at/l
			C	N	C	N	C	N		

Analyst's Signature ___________________________

DATA LOG FOR PARTICULATE P

Date Sampled: __________ Transect: __________ Station: __________

Date Analysed: __________ Analysing Laboratory: __________________

Time EST	Depth m	Volume Filtered ml	Blank OD	Std OD	Sample OD	PP µg at/l

Analyst's Signature ________________

DATA LOG FOR SUSPENDED SOLIDS

Date Sampled: ____________ Transect: _________ Station: _________

Date Analysed: ___________ Analysing Laboratory: _________________

Time EST	Depth m	Volume Filtered ml	Total wt mg	Filter wt mg	Solids wt mg	Solids mg/l

Analyst's Signature _________________

DATA LOG FOR SILICATE

Date Sampled: ____________ **Transect:** ____________ **Station:** _________

Date Analysed: ___________ **Analysing Laboratory:** ____________________

Time	Depth	Blank OD	Std OD	SiO_2 OD	SiO_2 µg atom/l

Analyst's Signature ____________________

DATA LOG FOR DIC/DOC

Date Sampled: ___________ Transect: ___________ Station: _________

Date Analysed: _________ Analysing Laboratory: ____________________

Time EST	Depth m	Volume Injected	DIC Blank	DOC Blank	DIC	DOC	DIC mg/l	DOC mg/l

Analyst's Signature ____________________

VI. QUALITY CONTROL

It is assumed that a valid sample has been properly taken, preserved, and delivered to he laboratory under careful quality control conditions. The laboratory control of analytical performance is but one vital link in obtaining valid data.

The two major measures of quality control are precision and accuracy. Precision refers to reproducibility among replicate observations, while accuracy is the degree of difference between observed and known or actual values.

a. Precision

Precision may be determined on duplicates of standards or actual samples (samples preferred), which can cover a wide range of concentrations and a variety of interfering materials.

b. Accuracy

Accuracy is a measure of how well the actual value may be determined. A known standard reference can be used or known amounts of the particular constituent can be added or spiked to the actual samples at various concentrations. It is desirable that amounts be added to a low concentration sample sufficient to double that concentration. Amounts added to intermediate concentrations should bring the final concentration in the sample up to approximately 75% of the upper limit. Further instructions regarding the actual mechanics of spiking may be found in the Appendices herein.

IT SHALL BE POLICY TO RUN A STANDARD OR SPIKE EVERY TENTH SAMPLE WHENEVER POSSIBLE. (See Appendices for Discussion of Method of Addition).

c. Charts

The precision and accuracy of analytical data produced in the laboratory can be monitored using control charts. These charts provide statistical ''proof'' of laboratory operations from day to day.

Two control charts are required for any given analytical procedure; the precision chart and the accuracy chart. Precision charts are constructed from duplicate sample analyses; accuracy charts are constructed from spiked sample analyses data.

There are three methods of charting data:

1. Shewhart
2. CuSum
3. Industrial

The selection of which chart to use is that of the analyst after using all three simultaneously to determine which best suit the sample types, concentration ranges, and precision and accuracy normally obtained in the laboratory. In all three techniques the construction of the control charts is based on the same initial raw data.

IT SHALL BE POLICY TO HAVE A SEPARATE PRECISION AND ACCURACY CONTROL CHART FOR EACH ROUTINE TEST IN THE LABORATORY. IT MAY BE NECESSARY TO HAVE MORE THAN ONE SET OF CONTROL CHARTS FOR A PARAMETER TO COVER MORE THAN ONE CONCENTRATION RANGE.

(1) Precision Charts

To construct a precision chart at least 20 sets of duplicate data are needed from routine sample for each parameter. It is necessary that these data be obtained under normal laboratory conditions, with routine analyst(s) using the same specified procedure.

The Shewhart method has been widely used since the 1920's by many industries. Inherent in the Shewhart approach is recognition of the basic assumption that variations exist in every method. Where these variations exist, either the instrument used to measure the process is not sensitive enough or the person making the measurement is not performing properly. The recorded difference between paired samples is compared to calculated acceptance limits.

The following information is used in the calculations of these limits:

(a) List the values obtained for paired sample ''Replicate 1'', ''Replicate 2'', and the difference between the two values: (d_i) or (R)

(b) Calculate the average difference ($\overline{R}$) by summing the list of d_i values and dividing by the number of sets of duplicates

$$\overline{R} = \frac{\Sigma\, d_i \text{ (or R)}}{n}$$

(c) Calculate the Upper Control Limit (UCL) on the difference according to the formula:

$UCL_R = D_4\overline{R}$ where D_4 factor is dependent on the number of observations, i.e., for

2, $D_4 = 3.27$

3, $D_4 = 2.58$

4, $D_4 = 2.28$

(d) Calculate Upper Warning Limit (UWL) on the difference according to the formula:

$$UWL_R = 2/3(D_4\overline{R} - \overline{R}) + \overline{R}$$

For duplicates, this equation reduces to:

$$UWL_R = 2.51\ \overline{R}$$

This UWL corresponds to the 95% confidence limit.

(e) The standard deviation can also be calculated from this information according to:

PRECISION CHART

Parameter: ________

Date : ________

Analyst : ________

S =

UWL =

UCL =

n	Sample No.	Date	A_1	A_2	A_1-A_2	A_1+A_2	$x=\left\|\frac{A_1-A_2}{A_1+A_2}\right\|$	$\left\|\,\|x\|-\bar{x}\right\|$	$(x-\bar{x})^2$
1									
2									
3									
4									
5									
6									
7									
8									
9									
10									
11									
12									
13									
14									
15									
16									
17									
18									
19									
20									
21									
22									
23									
24									
25									

$$\bar{X} = \frac{1}{n} \sum_{n=1}^{25} \left|\frac{A_1-A_2}{A_1+A_2}\right|$$

$$S = \sqrt{\frac{\sum\limits_{n=1}^{25} (X-\bar{X})^2}{n-1}}$$

2S = Upper Warning Limit (UWL)

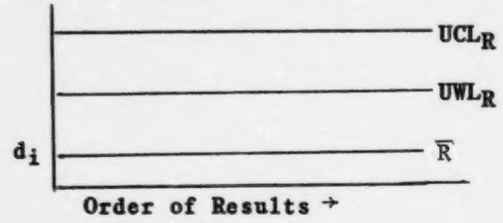
3S = Upper Control Limit (UCL)

$$s_d^2 = \frac{\dfrac{\Sigma\, d_i^2 - \Sigma\, (d_i)^2}{n}}{n - 1}$$

$$s_d = \sqrt{s_d^2}$$

This value should be checked against the published s_d for the stated method (at similar concentration levels). Samples of s_d values for various methods at AFO are presented later in this section.

(f) The plot form appears as below:



d_i is plotted daily for each pair of sample results, new charts are constructed after 25 sets. Data is ''in-control'' if the point falls below the UCL_R. See ''Corrective Procedures'' for response to ''out-of-control''. See also the attached Tally Sheet which is used to record the data and when completed is used to construct the next chart.

The CuSum method is an extension of Shewhart. Shewhart examines the difference between two points relative to a predetermined limit but fails to account for time as an error factor, CuSum attempts to monitor ''trends'' by summing the cumulative differences with respect to time. Specifically, each d_i is squared and added to the previous summation and compared to limits specified for that point in time.

The following information is used to construct a CuSum chart:

(a) List values '1' and '2' as in Shewhart and calculate d_i

(b) Square d_i

(c) Add d_i^2 to previous d_i^2 for $\Sigma\ (d_i^2)$

(d) After 20 or more sets, divide $\Sigma\ (d_i^2)$ by $n-1 = s_d^2$

(e) Calculate Upper Limit for M sets = 0 (beginning of chart):

$$UL_{(M=0)} = 4.07(s_d^2)$$

(f) Calculate Lower Limit for M sets = 0

$$LL_{(M=0)} = -4.07(s_d^2)$$

(g) Calculate Upper Limit for M sets = 25 (end of chart):

$$UL_{(M=25)} = 4.07(s_d^2) + 25(.94)(s_d^2)$$

(h) Calculate Lower Limit for M sets = 25

$$LL_{(M=25)} = -4.07(s_d^2) + 25(.94)(s_d^2)$$

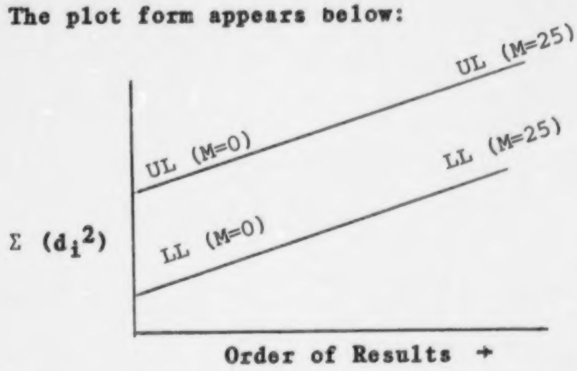
NOTE: The above equations are based on the following assumptions used in reducing the original equations

$\alpha = .15$ $\quad s_o^1 = .8$

$\beta = .15$ $\quad s_o^2 = 1.2$

If these values are changed, consult AQC Manual, Chapter 6 for appropriate equations.

(i) The plot form appears below:



$\Sigma\ (d_i^2)$ is plotted daily for each pair of sample resuts, new charts are constructed after 25 sets. Data is ''in-control'' if it falls between the Upper Limit Line and the Lower Limit Line. See ''Corrective Procedures'' for response to ''out-of-control''. AFO Tally sheet follows which is used for monitoring data and when completed is used to construct the next chart.

The third method of plotting, the Industrial chart, attempts to compensate for yet another factor affecting precision and accuracy: both may change over a specified concentration range and the same error (i.e., 0.1 mg/1) will be more or less significant depending on the concentration level of the sample showing such an error. For example, at the 0.5 level, 0.1 represents a 20% error, while at the 1.5 level, 0.1 represents only a 7% error. To truly determine if an error is significant requires that consideration be given to concentration; the Industrial chart attempts to do this. To a certain extent, this may remove the necessity of more than one chart for different concentration levels. The following information is used for Industrial chart calculations:

(a) List values obtained for paired set as A_1 and A_2

(b) Calculate $|A_1 - A_2|$

(c) Calculate $A_1 + A_2$

(d) Calculate $\left|\frac{A_1 - A_2}{A_1 + A_2}\right|$; hereinafter called 'X'

(e) After 20 - 25 sets, calculate $\bar{X}$, as follows:

$$\Sigma \left|\frac{A_1 - A_2}{A_1 + A_2}\right| \Big/ n$$

, where n = number of paired sets

(f) For each pair, calculate $X - \bar{X}$

(g) Calculate $(|X - \bar{X}|)^2$

(h) Calculate $\Sigma(|X - \bar{X}|)^2$

(i) Calculate s_d where

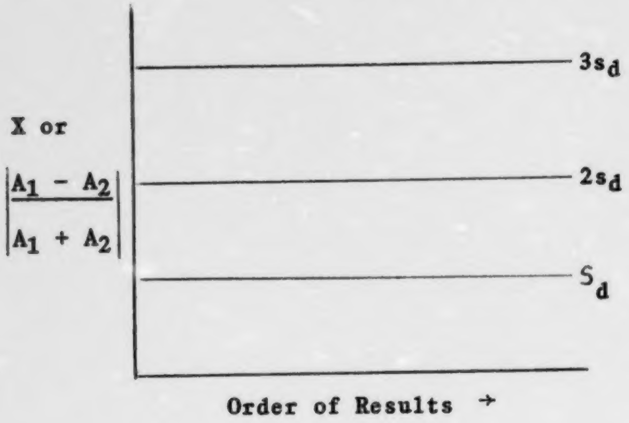
$$s_d = \sqrt{\frac{\Sigma(X - \bar{X})^2}{n-1}}$$

then Upper Warning Limit = $2s_d$

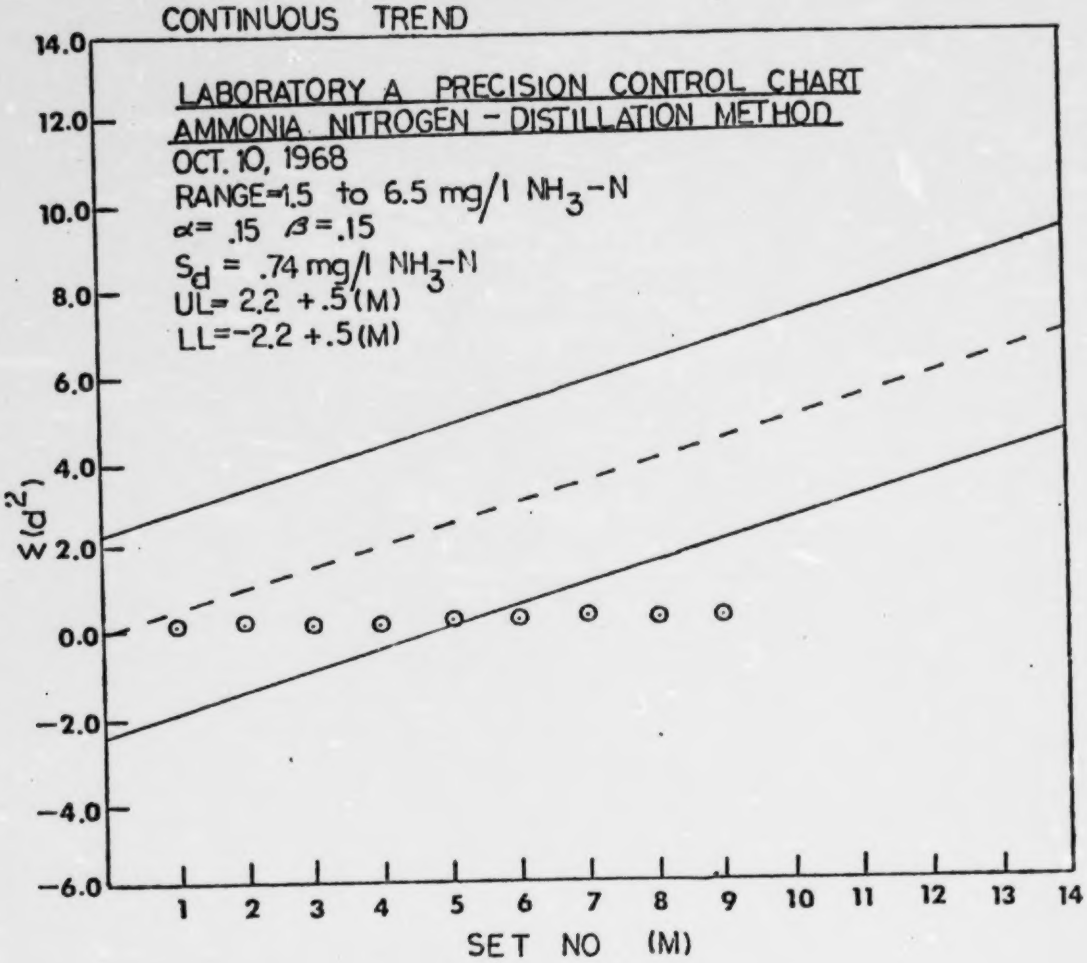
Upper Control Limit = $3s_d$

After chart is constructed, plot 'X' or $\left|\frac{A_1 - A_2}{A_1 + A_2}\right|$ daily for each pair.

(j) The plot form appears below:



'X' is plotted daily, new charts are constructed after 25 sets. Data is ''in-control'' if it falls within 3 s_d See ''Corrective Procedures'' for response to ''out-of-control''. AFO Tally Sheet follows which is used for monitoring data and when completed is used to construct new chart.



CORRECTIVE PROCEDURES:

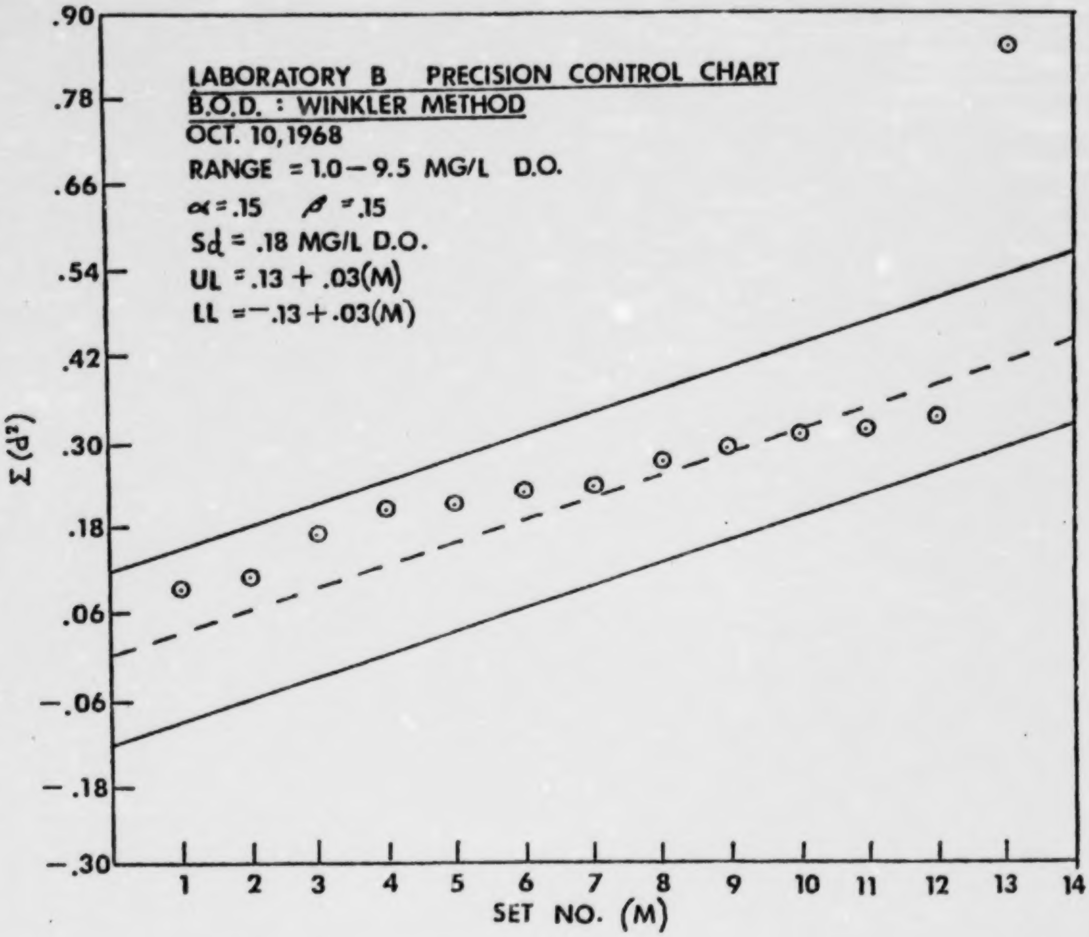
1 Continue analysis beyond sample set 6 through series of samples unless trend changes

2 Assume increased efficiency

3 Construct new chart on recent data

4 Begin plotting $\Sigma(d^2)$ at sample set 1

5 Observe analyst for false reporting



CORRECTIVE PROCEDURES:

1 Stop analysis at sample set 13

2 Locate cause or assume chance causes

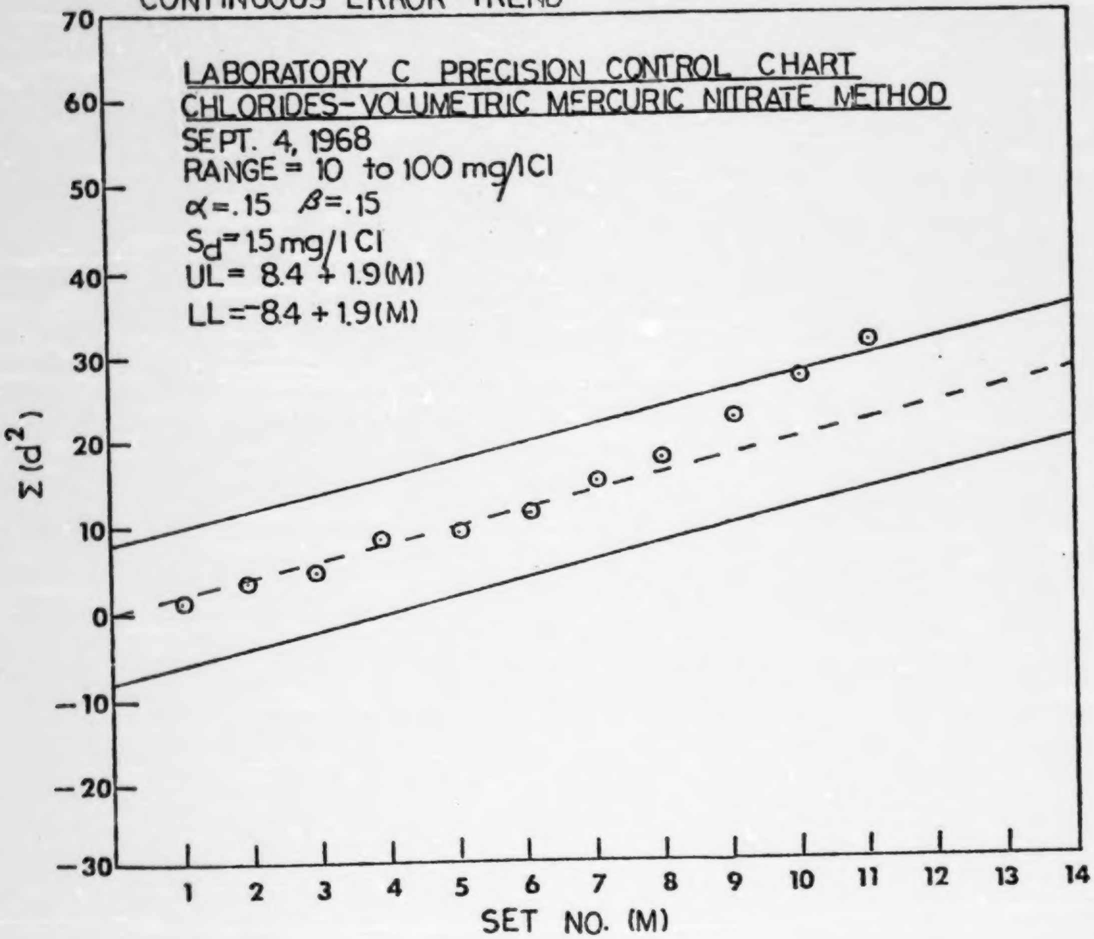
3 Correct problem if possible

4 Samples cannot be rerun on BOD – reject all data between sample sets 12 and 13

5 If duplicates are run on all samples – reject sample set 13

6 Begin plotting $\Sigma(d^2)$ at sample set 1

CONTINUOUS ERROR TREND



CORRECTIVE PROCEDURES:

1 Analysis could have been stopped at sample set 9 or 10

2 Analysis definitely stopped at sample set 11

3 Locate cause

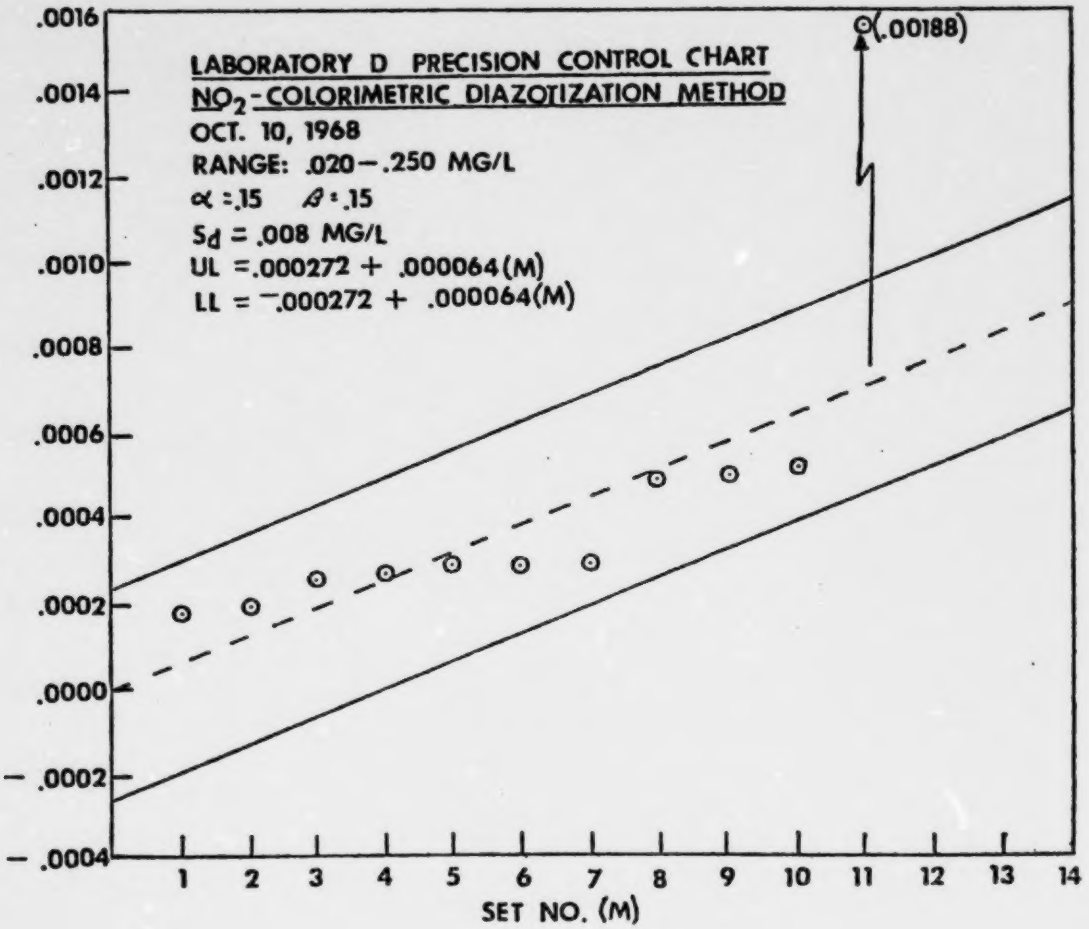
4 Correct problem

5 If analysis stopped at sample set 9 - rerun samples between sample sets 8 and 9

6 If analysis stopped at sample set 10 - rerun samples between sample sets 9 and 10

7 If analysis stopped at sample set 11 - rerun samples between sample sets 10 and 11

8 In any case begin plotting $\Sigma(d^2)$ at sample set 1



CORRECTIVE PROCEDURES:

1 Stop analysis at sample set 11

2 Locate cause or assume chance causes

3 Correct problem

4 Rerun samples between sample sets 10 and 11

5 Begin plotting $\Sigma(d^2)$ at sample set 1

SUMMARY:

Shewhart - based on simple differnce (d_i) compared to average difference calculated from past data

Limitations:

1. Does not readily show trends (i.e., reagent decomposition)
2. Does not account for variations in precision and accuracy over working concentrated range

CuSum - based on simple difference squared (d_i^2) and added to previous difference

Limitations:

1. Allows one sample to greatly affect other values depending on its placement when plotted
2. Same as '2' above

Industrial - based on ''significance'' of difference depending on the concentration level at which the difference occurs

Limitations:

1. Does not readily show trends
2. Calculations more cumbersome and time-consuming

(2) Accuracy Charts

To construct an accuracy chart at least 20 sets of spiked sample data are needed from routine samples for a given parameter. It is necessary that these data be obtained under normal laboratory conditons, with routine analyst(s) using the specified procedure.

Any of the three charting methods as previously described may be used. Instead of duplicates, however, lists should be made as below:

Shewhart Precision -	Replicate 1	Replicate 2
	↓	↓
Accuracy -	Actual or True Value	Recovered Value

CuSum: same as above

Industrial Precision -	A_1	A_2
	↓	↓
Accuracy -	Actual or True Value	Recovered Value

Calculations are the same as outlined earlier. In addition, a column can be added to the accuracy charts for computing % bias (% error) according to:

ACCURACY CHART

Parameter: ____________

Date : ____________

Analyst : ____________

S =

UWL =

UCL =

n	Sample No.	True Value A_1	Recover. Value A_2	A_1-A_2	A_1+A_2	$x=\left\|\frac{A_1-A_2}{A_1A_2}\right\|$	$\left\|\,\|X\|-\bar{X}\right\|$	$(X-\bar{X})^2$	% Recovery	% Error
1										
2										
3										
4										
5										
6										
7										
8										
9										
10										
11										
12										
13										
14										
15										
16										
17										
18										
19										
20										
21										
22										
23										
24										
25										

$$\bar{X} = \frac{1}{n} \sum \left|\frac{A_1-A_2}{A_1+A_2}\right|$$

$$S = \sqrt{\frac{\sum_{n=1}^{25} (X-\bar{X})^2}{n-1}}$$

2S = Upper Warning Limit (UWL)

3S = Upper Control Limit (UCL)

$$\frac{\text{True} - \text{Recovered}}{\text{True}} \times 100 = \%\ \text{bias}$$

Standard deviation and % bias are the expressions used in most manuals to describe precision and accuracy. By computing these values, AFO can compare its performance to that of published methods. The values for precision and accuracy at AFO are presented later in this section and also with each method in the companion methods manual.

Sample Tally Sheets follow.

(3) Corrective Procedures

(a) Reasons for being ''out-of-control''

(i) Precision - Analyst (primary source)

- Nature of sample (homogeneity and ability to separate sample into two or more equal parts and allow true duplicate analysis; samples which contain oil or clumps of material are almost impossible to duplicate)
- Glassware contamination
- Increased efficiency or false data reporting
- Chance causes - check everything else first before assuming ''chance''

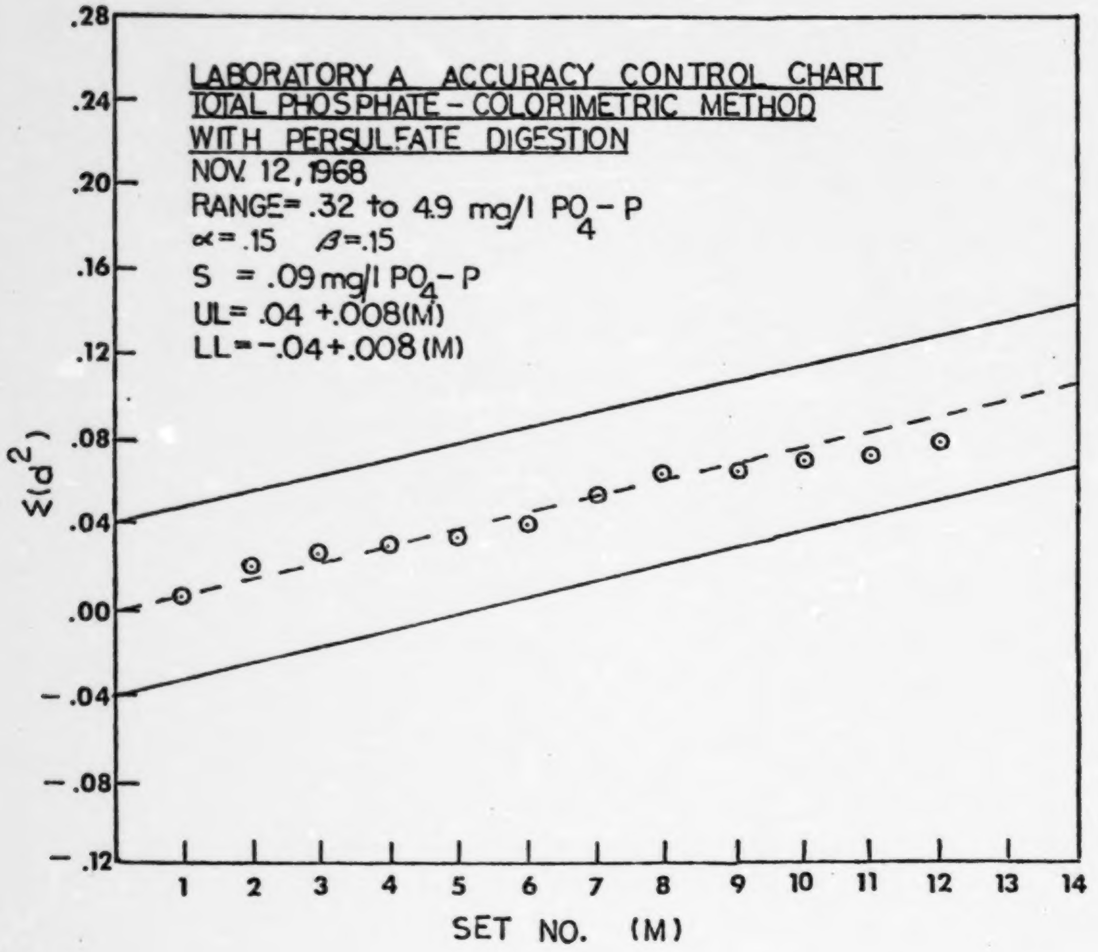
NOTE: Not concerned with reagents or

instrumentation in precision since analyzing a duplicate sample would duplicate any reagent or instrument error

(ii) Accuracy - Analyst
- Glassware contamination
- Contaminated reagents
- Instrumentation
- Sample interference with spiked material
- Contaminated laboratory atmosphere
- Increased efficiency or false data reporting

(iii) Response to ''out-of-control''

The attached examples should serve as a guideline in handling out-of-control samples. If there are questions, consult AQC Officer. Any out-of-control data that is ultimately reported should be appropriately flagged.

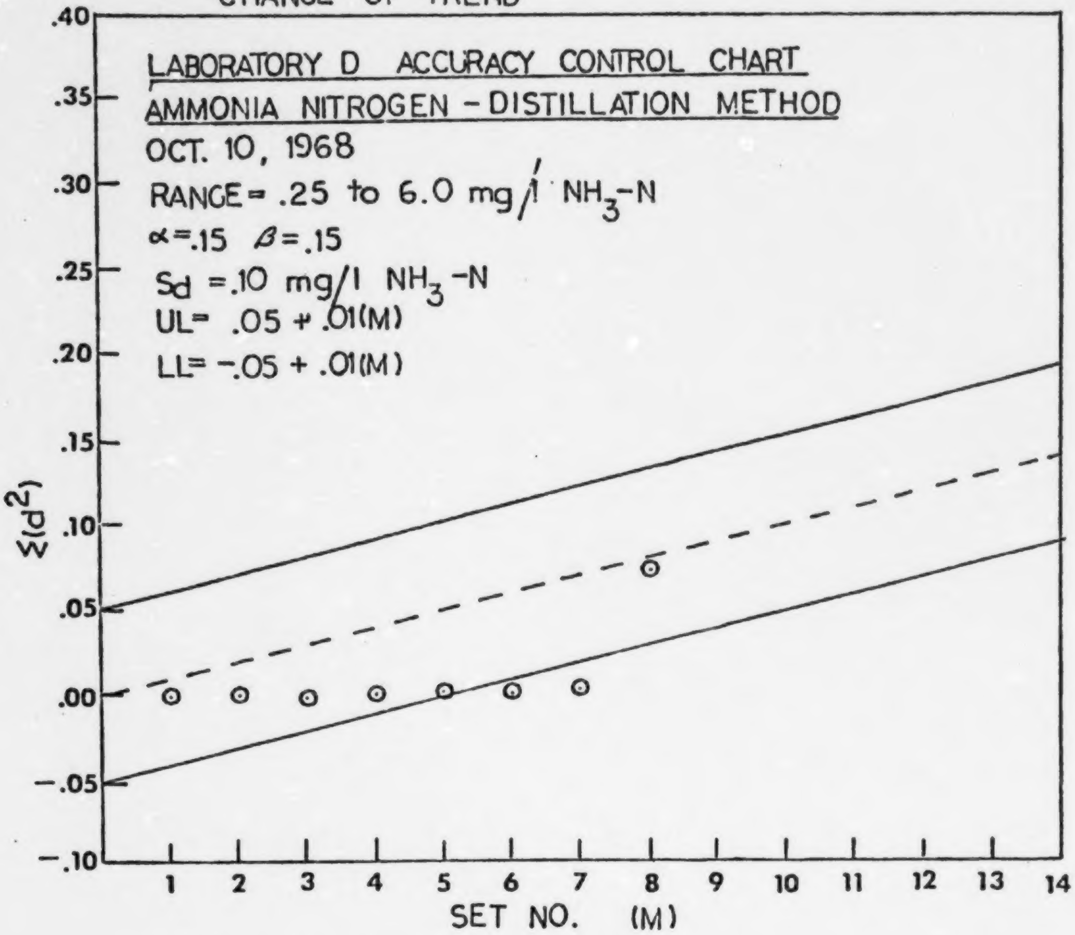


CORRECTIVE PROCEDURES:

1 No problems

2 Continue analysis

CHANGE OF TREND



CORRECTIVE PROCEDURES:

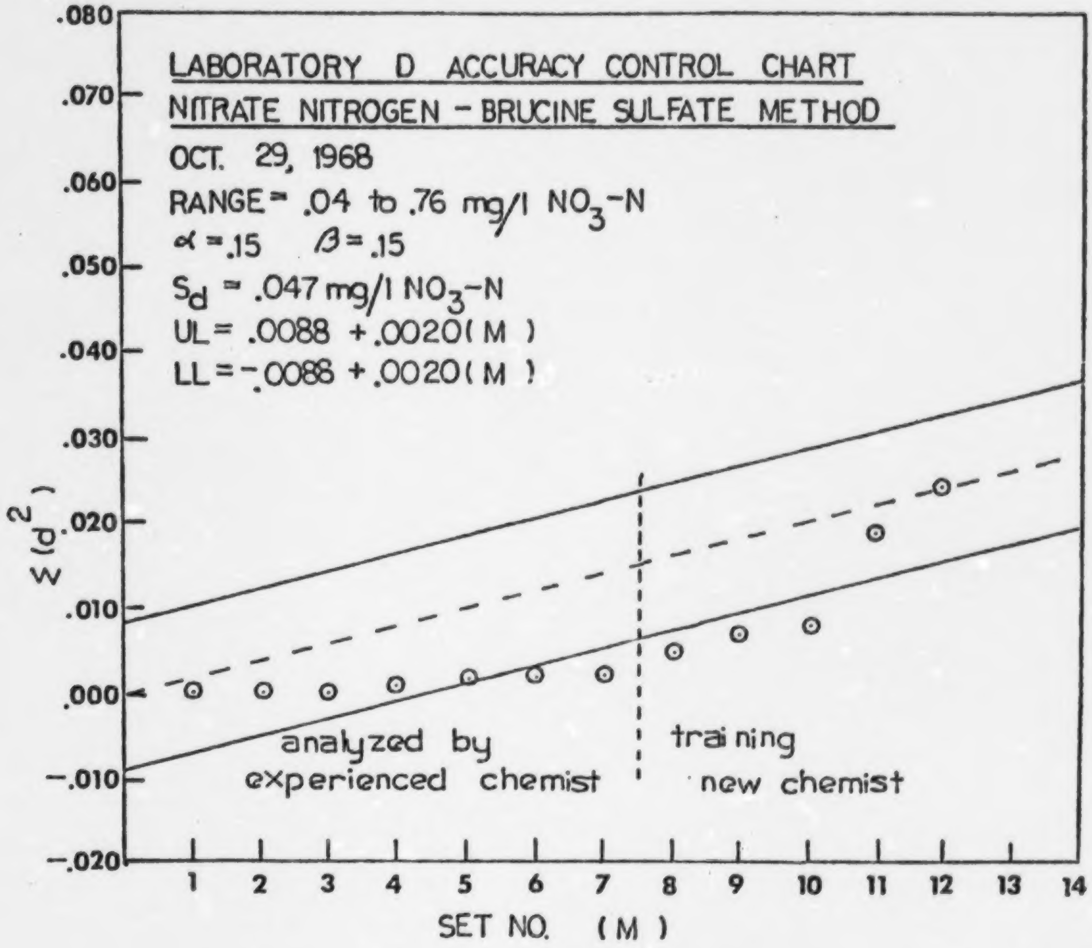
1 Stop charting at sample set 8

2 Begin new chart by plotting $\Sigma(d^2)$ of sample set 8 at sample set 1

3 Observe operations for possible problems

4 Continue analysis with caution

EFFECT OF TRAINING



CORRECTIVE PROCEDURES:

1 Samples 1 through 7 analyzed by experienced chemist

2 Training of inexperienced chemist began at sample set 8

3 Training continued through sample set 11

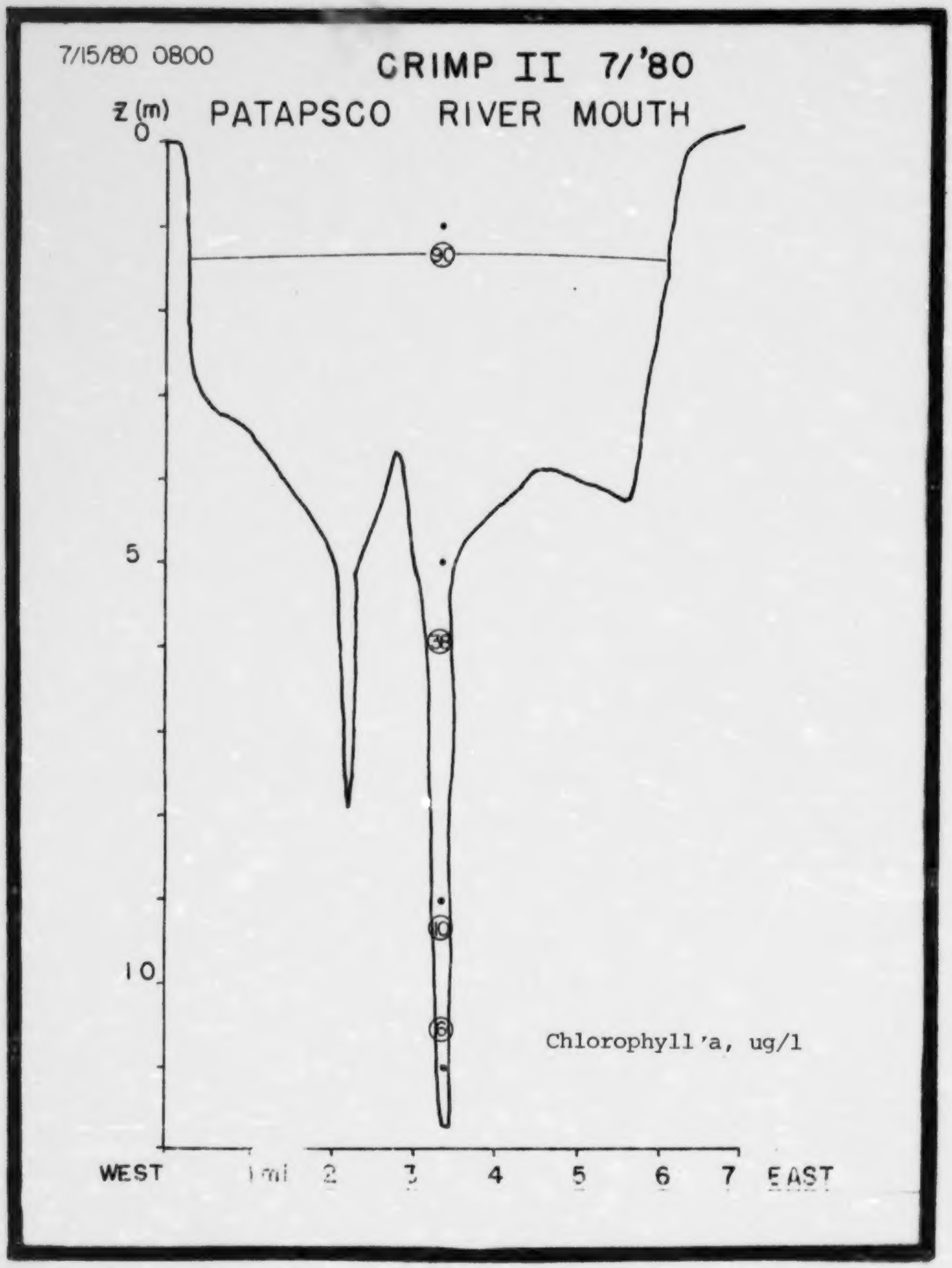
4 Inexperienced chemist took complete control on sample set 12

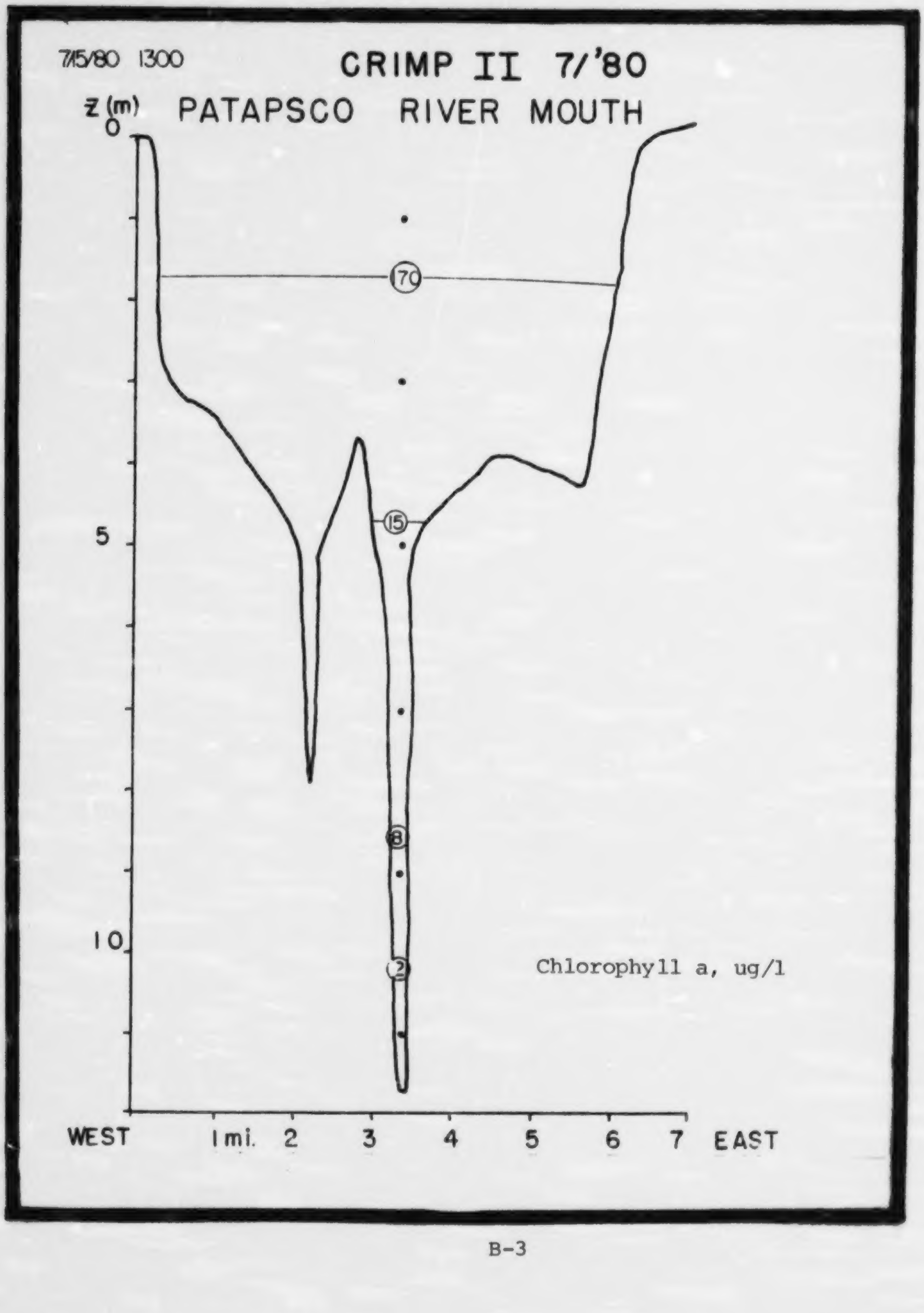
APPENDIX B

The appendix contains representitive graphical summaries of some of the data collected during the bay-wide study in July 1980. Sampling stations are shown in Figure 1. The collection and analytical methods are discussed in Appendix A, the quality assurance plan for the study. Units for the data displayed here are:

Chlorophyll *a*	ug/liter
Salinity	mg/liter
Particulate Carbon	ug atom/liter
Suspended Solids	mg/liter
Dissolved Oxygen	mg/liter

The contour lines are drawn through vertical data distributions which may not be complete because measurements were made only at depths adjacent to current meters. In most of the river mouths there was only one vertical profile so contours were drawn straight across the mouth. For this study, it was decided to emphasize temporal variations so data were collected in each location twice each day for eight days. At the bay mouth data were taken at 5 stations every three hours for 36 hours and twice daily thereafter.

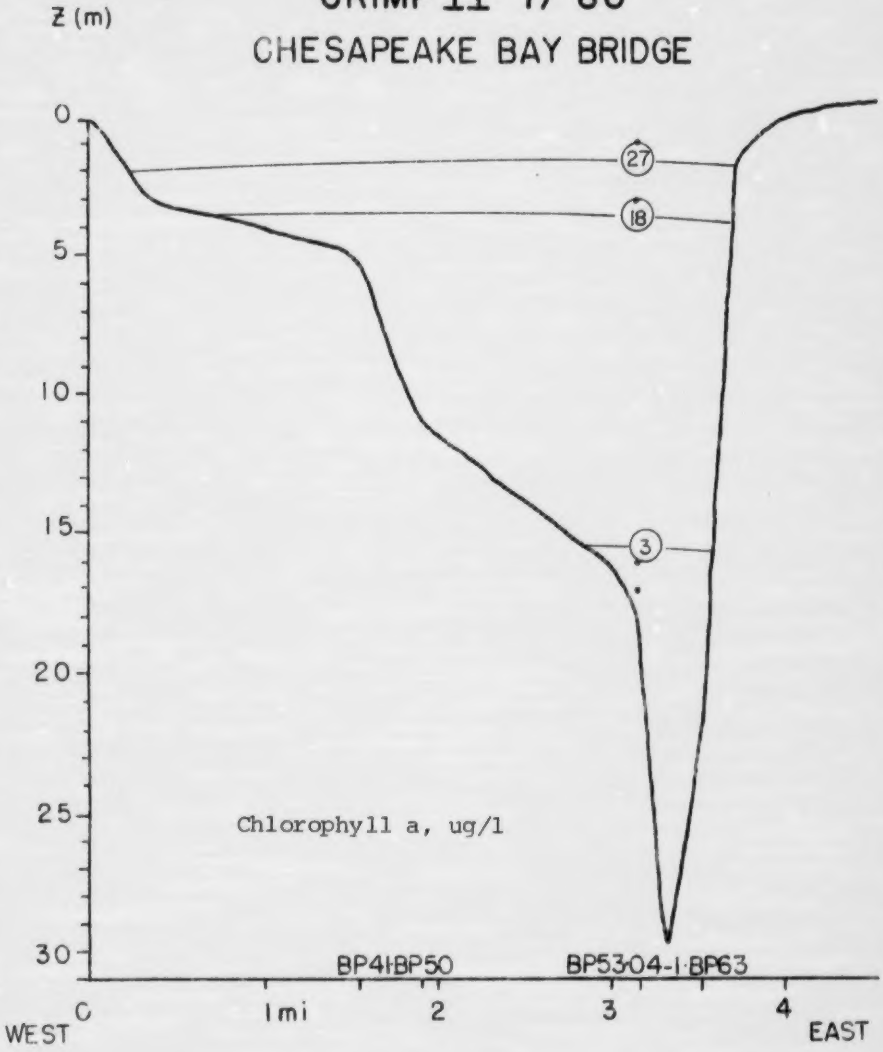

7/15/80 0800
CRIMP II 7/'80
PATAPSCO RIVER MOUTH
z (m)
0
5
10
90
36
10
6
Chlorophyll a, ug/l
WEST
1 mi
2
3
4
5
6
7
EAST

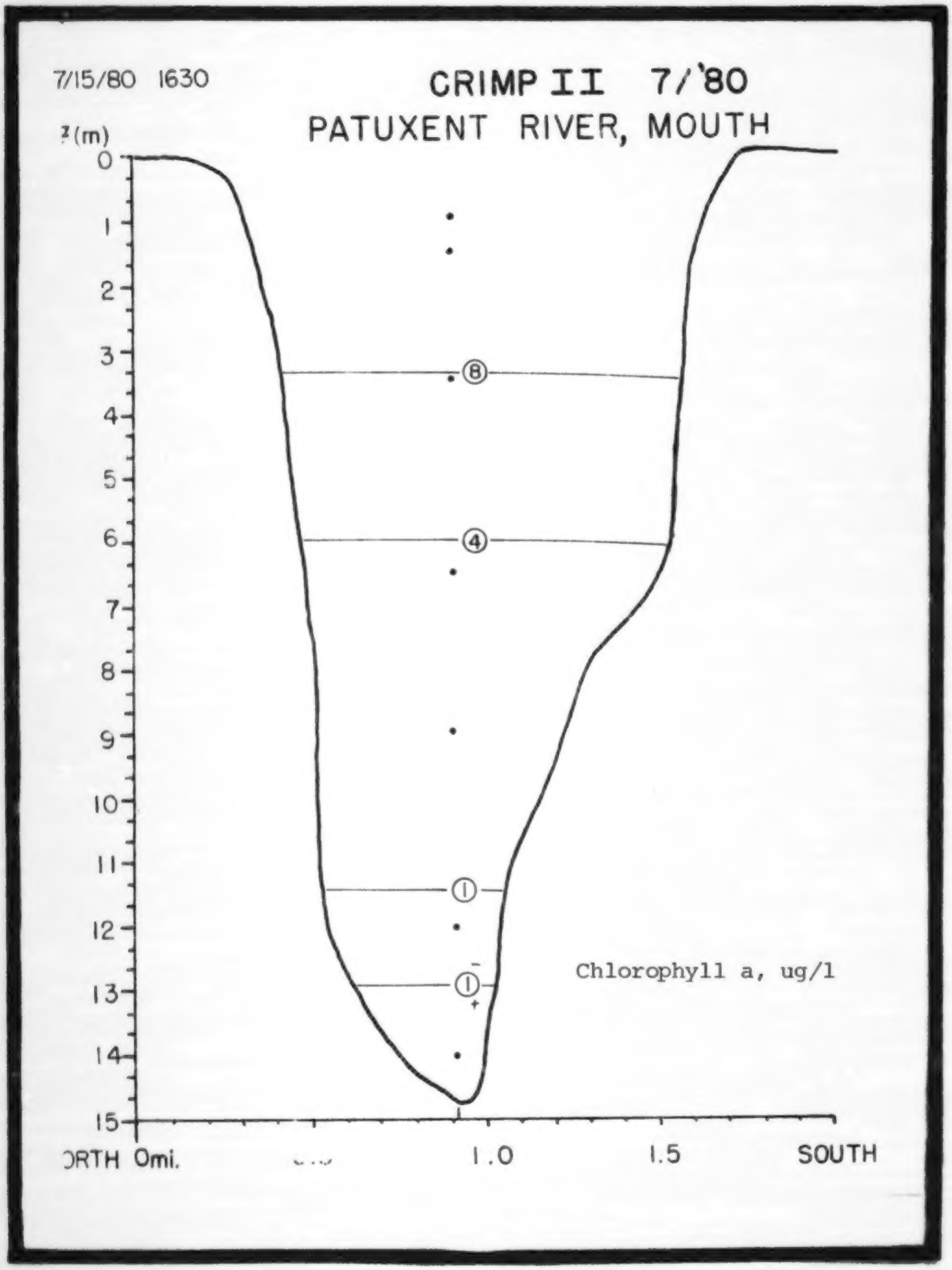

7/15/80 1300
CRIMP II 7/'80
PATAPSCO RIVER MOUTH
z (m)
0
5
10
70
15
8
2
WEST
1 mi.
2
3
4
5
6
7
EAST
Chlorophyll a, ug/l

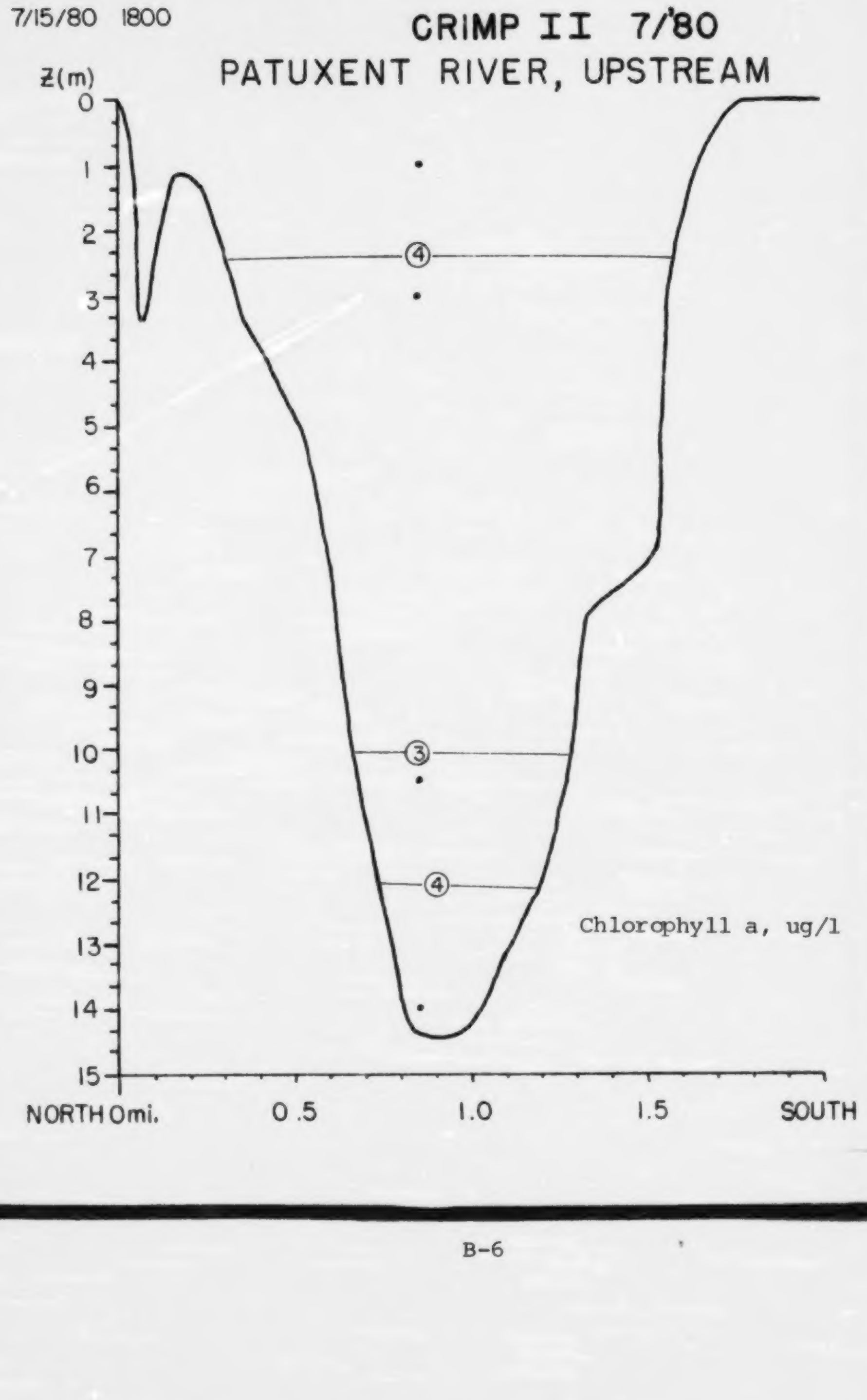
7/15/80 0805

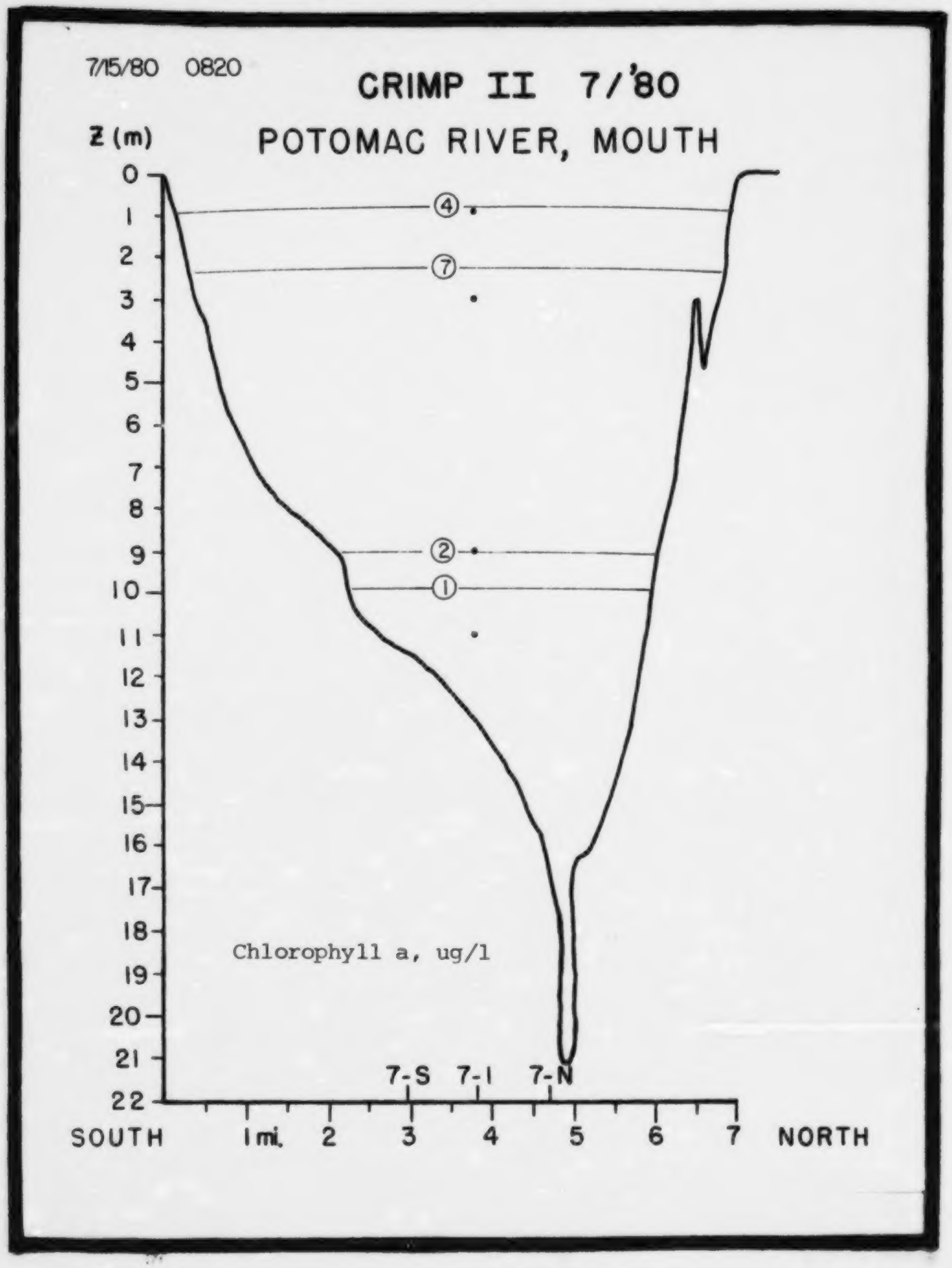
CRIMP II 7/'80

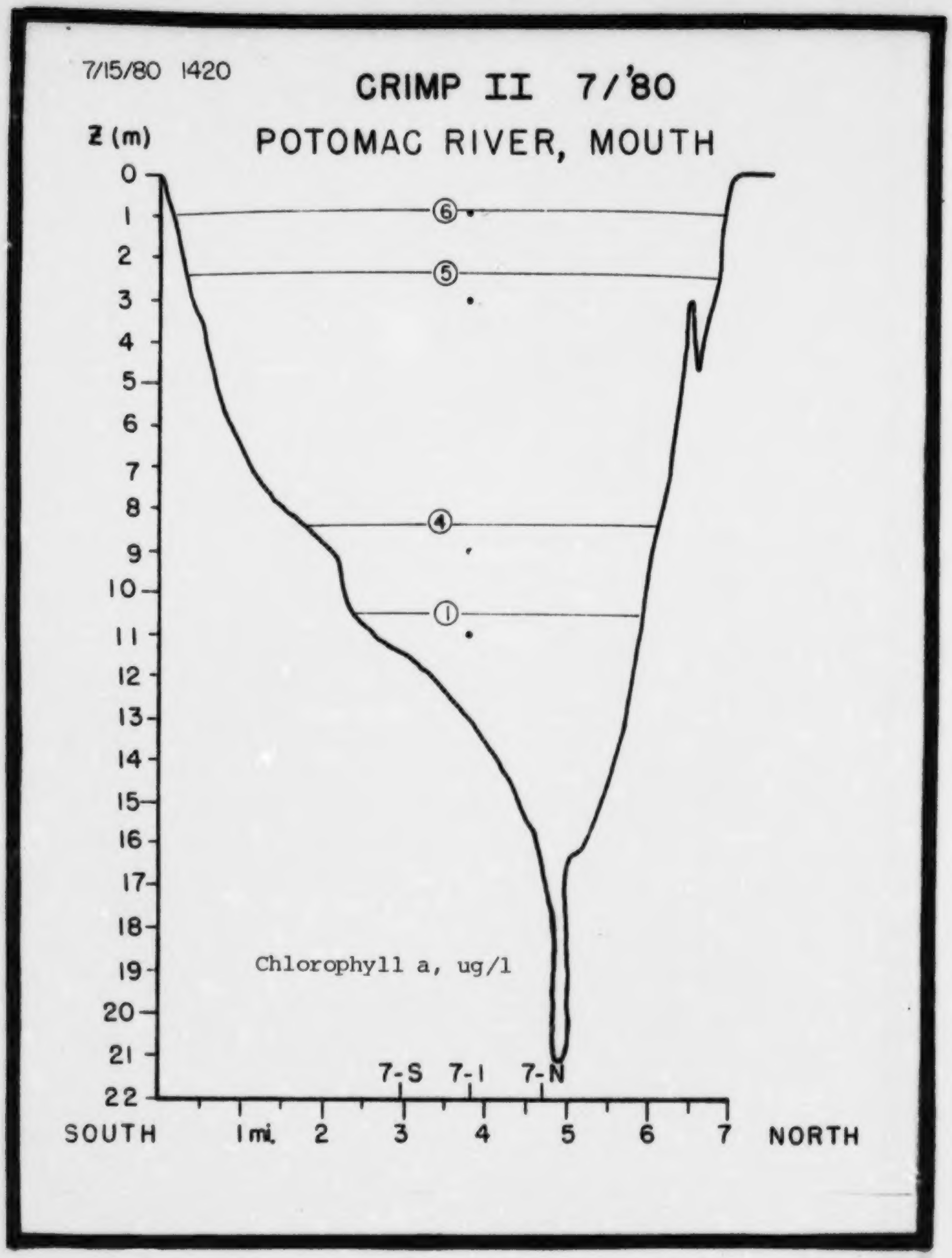
CHESAPEAKE BAY BRIDGE

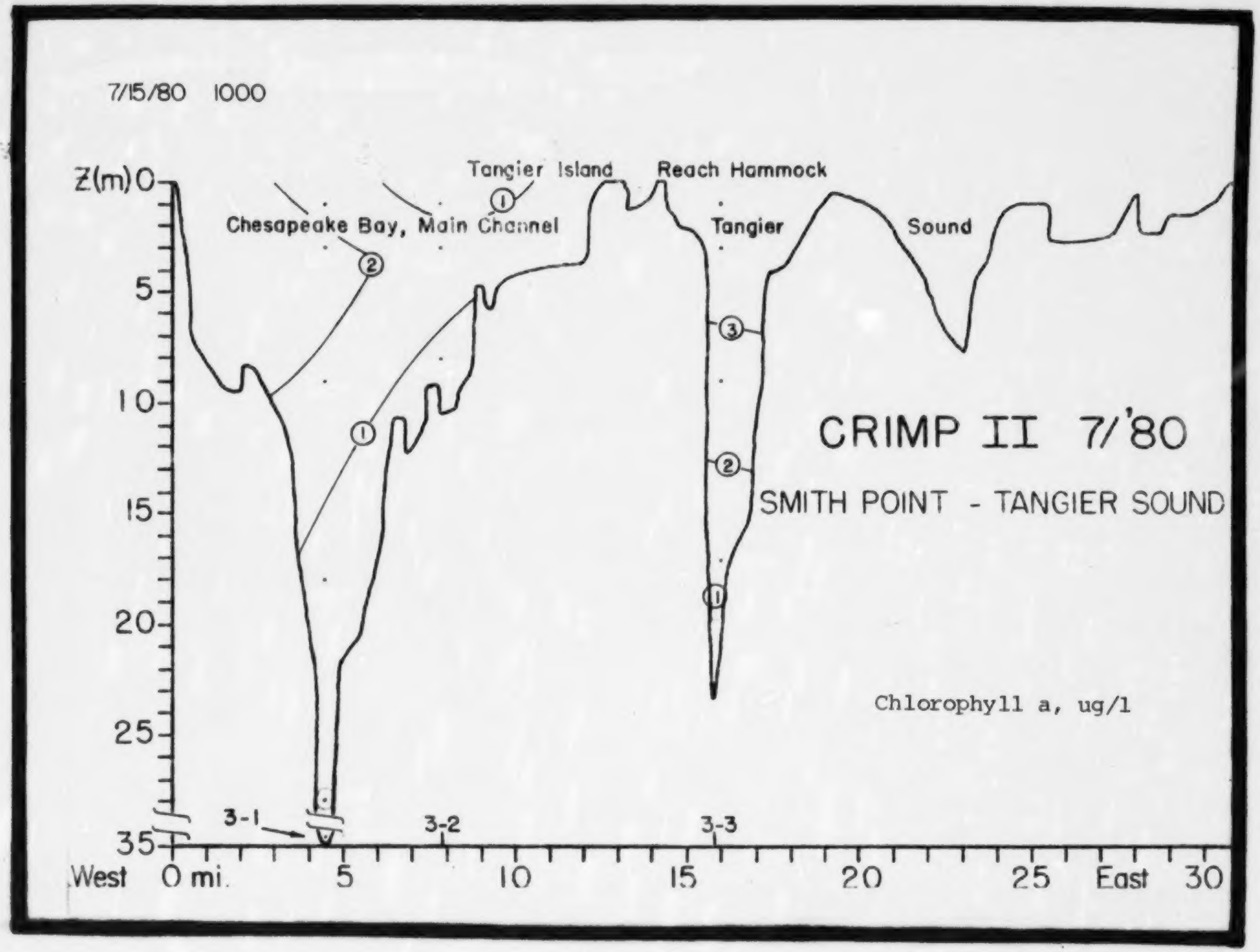


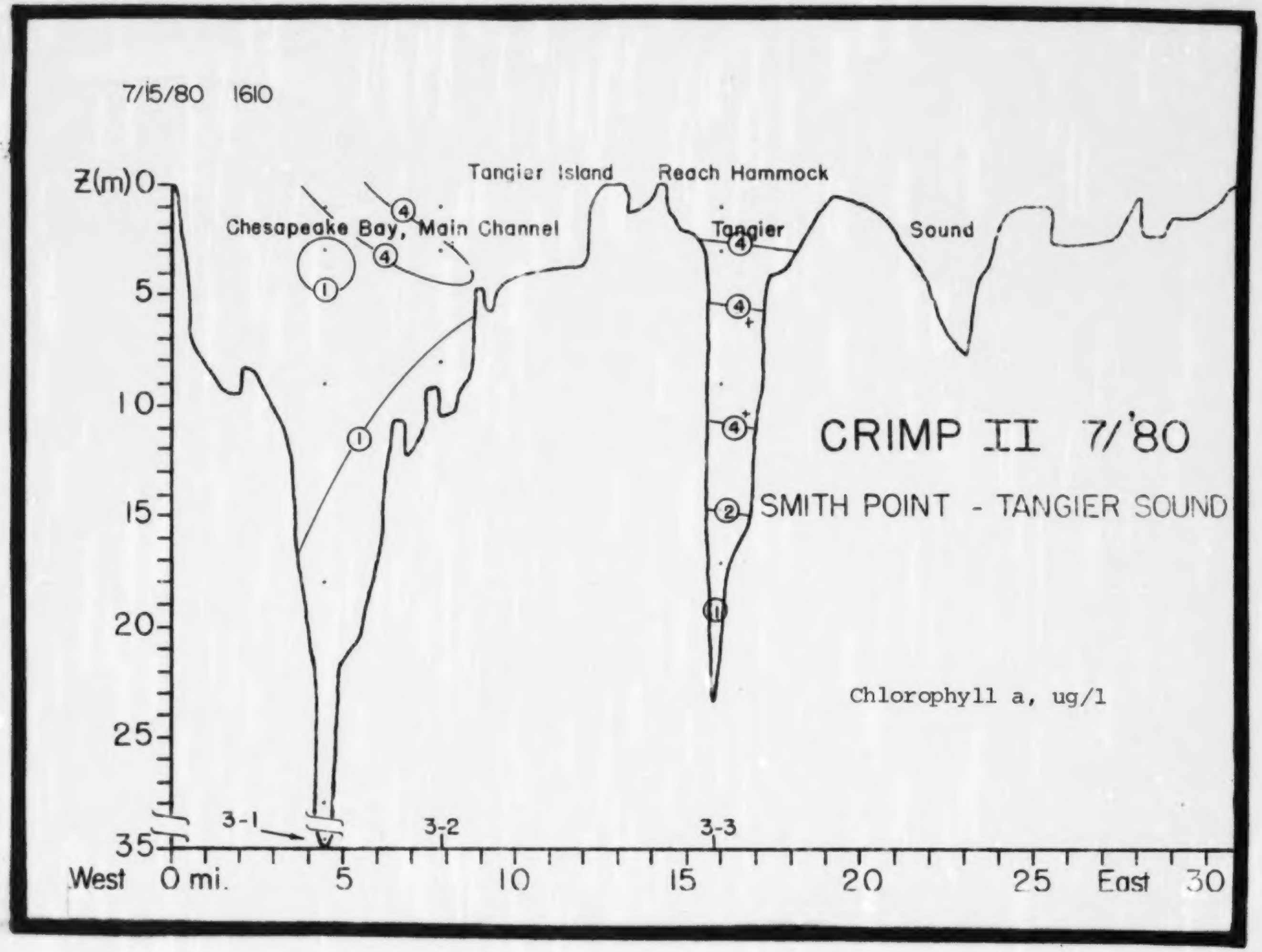

7/15/80 1630
CRIMP II 7/'80
PATUXENT RIVER, MOUTH
z(m)
0
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
8
4
1
1
Chlorophyll a, ug/l
ORTH 0mi.
1.0
1.5
SOUTH

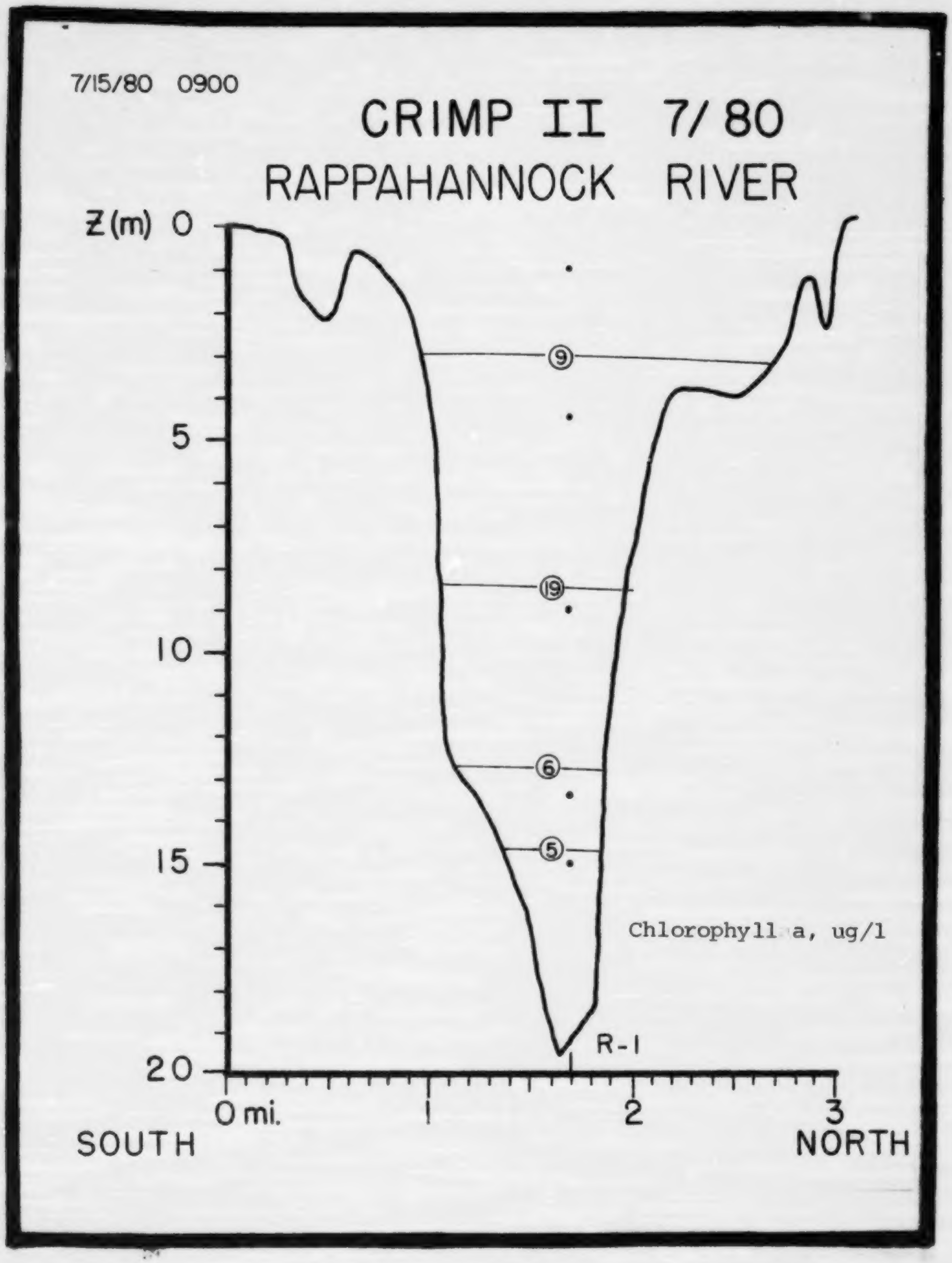

7/15/80 1800
CRIMP II 7/'80
PATUXENT RIVER, UPSTREAM
Z(m)
0
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
④
③
④
Chlorophyll a, ug/l
NORTH 0mi.
0.5
1.0
1.5
SOUTH

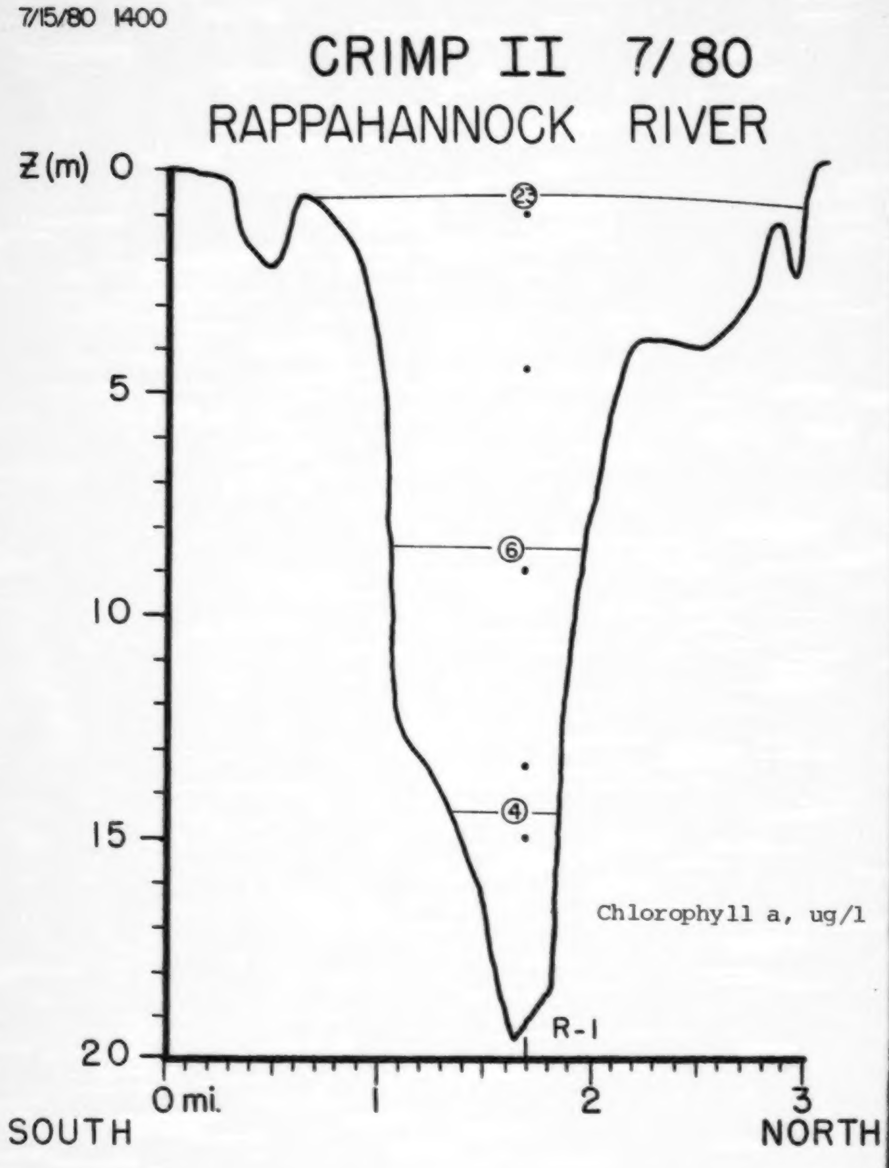

7/15/80 0820
CRIMP II 7/'80
POTOMAC RIVER, MOUTH
Z (m)
0
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
④
⑦
②
①
Chlorophyll a, ug/l
7-S
7-1
7-N
SOUTH
1 mi.
2
3
4
5
6
7
NORTH


7/15/80 1420
CRIMP II 7/'80
POTOMAC RIVER, MOUTH
Z (m)
0
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
⑥
⑤
④
①
Chlorophyll a, ug/l
7-S
7-1
7-N
SOUTH
1 mi.
2
3
4
5
6
7
NORTH


7/15/80 1000
CRIMP II 7/'80
SMITH POINT - TANGIER SOUND
Chlorophyll a, ug/1
Z(m)
0
5
10
15
20
25
35
West
0 mi.
5
10
15
20
25
East
30
Chesapeake Bay, Main Channel
Tangier Island
Reach Hammock
Tangier
Sound
3-1
3-2
3-3
①
②
③


7/15/80 1610
Z(m) 0
5
10
15
20
25
35
West
0 mi.
5
10
15
20
25
East
30
3-1
3-2
3-3
Tangier Island
Reach Hammock
Chesapeake Bay, Main Channel
Tangier
Sound
①
②
④
CRIMP II 7/'80
SMITH POINT - TANGIER SOUND
Chlorophyll a, ug/l

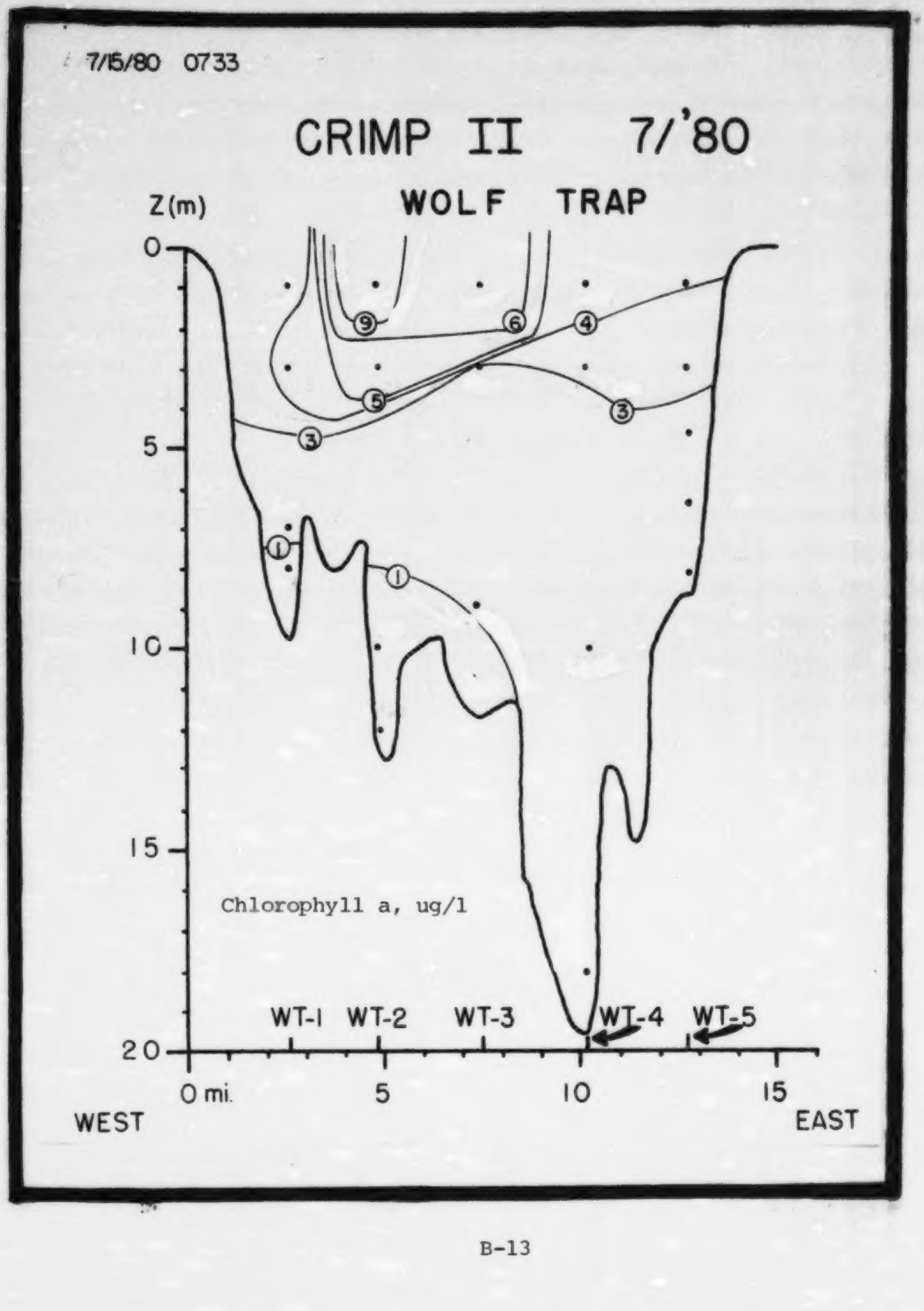

7/15/80 0900
CRIMP II 7/80
RAPPAHANNOCK RIVER
Z(m)
0
5
10
15
20
⑨
⑲
⑥
⑤
Chlorophyll a, ug/l
R-1
0 mi.
1
2
3
SOUTH
NORTH



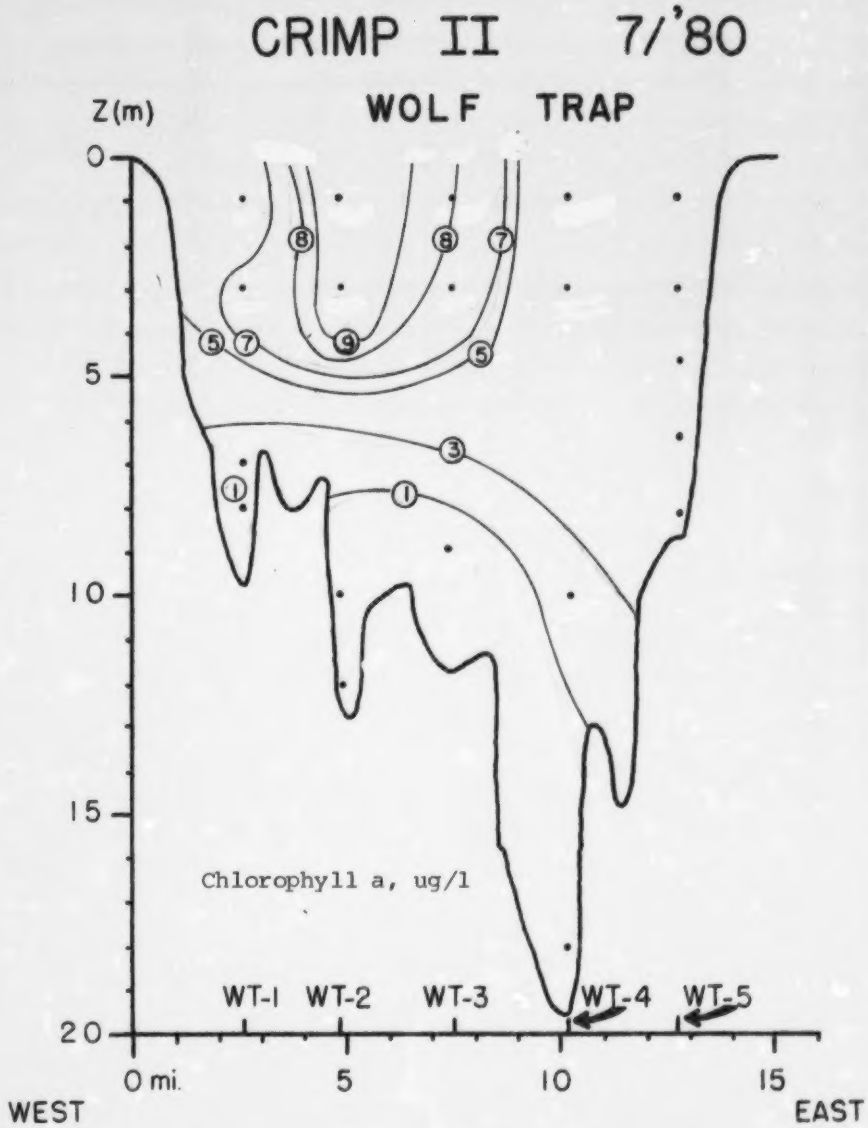
7/15/80 1400

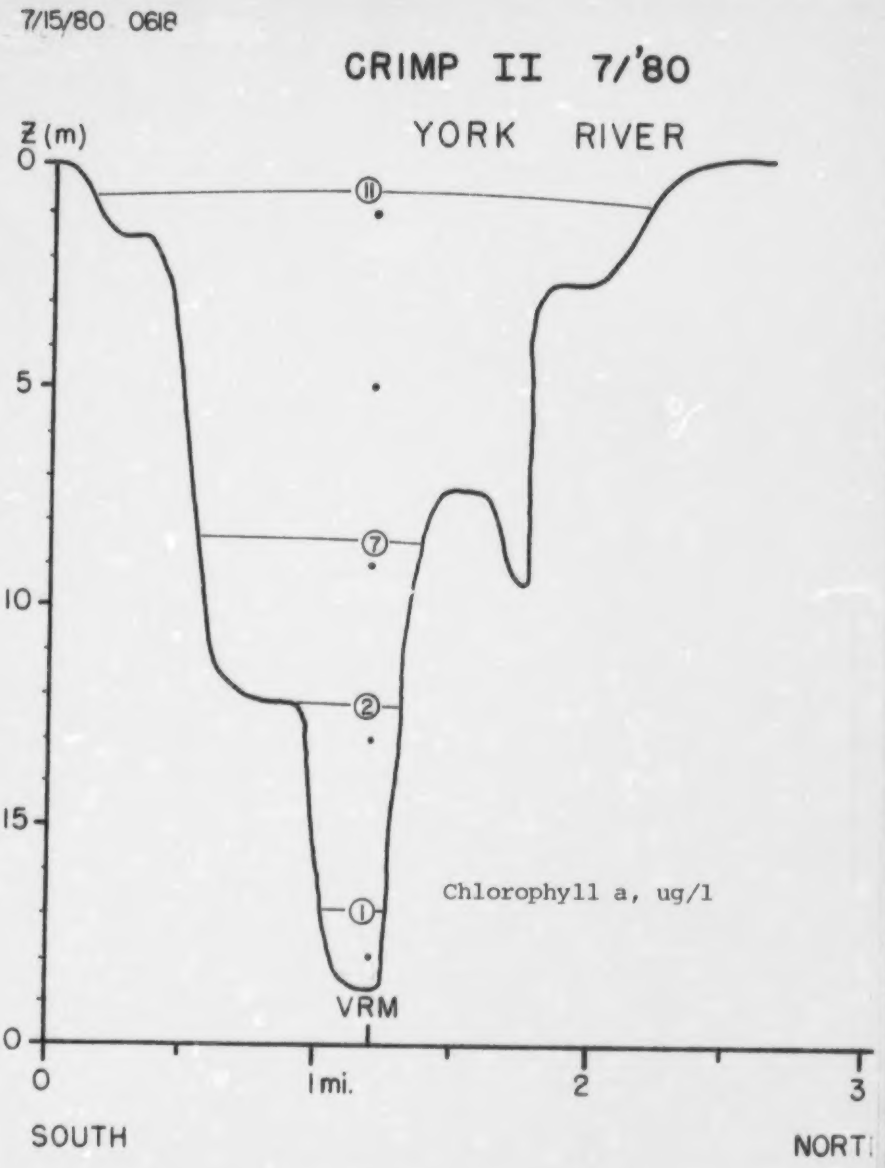
CRIMP II 7/80
RAPPAHANNOCK RIVER

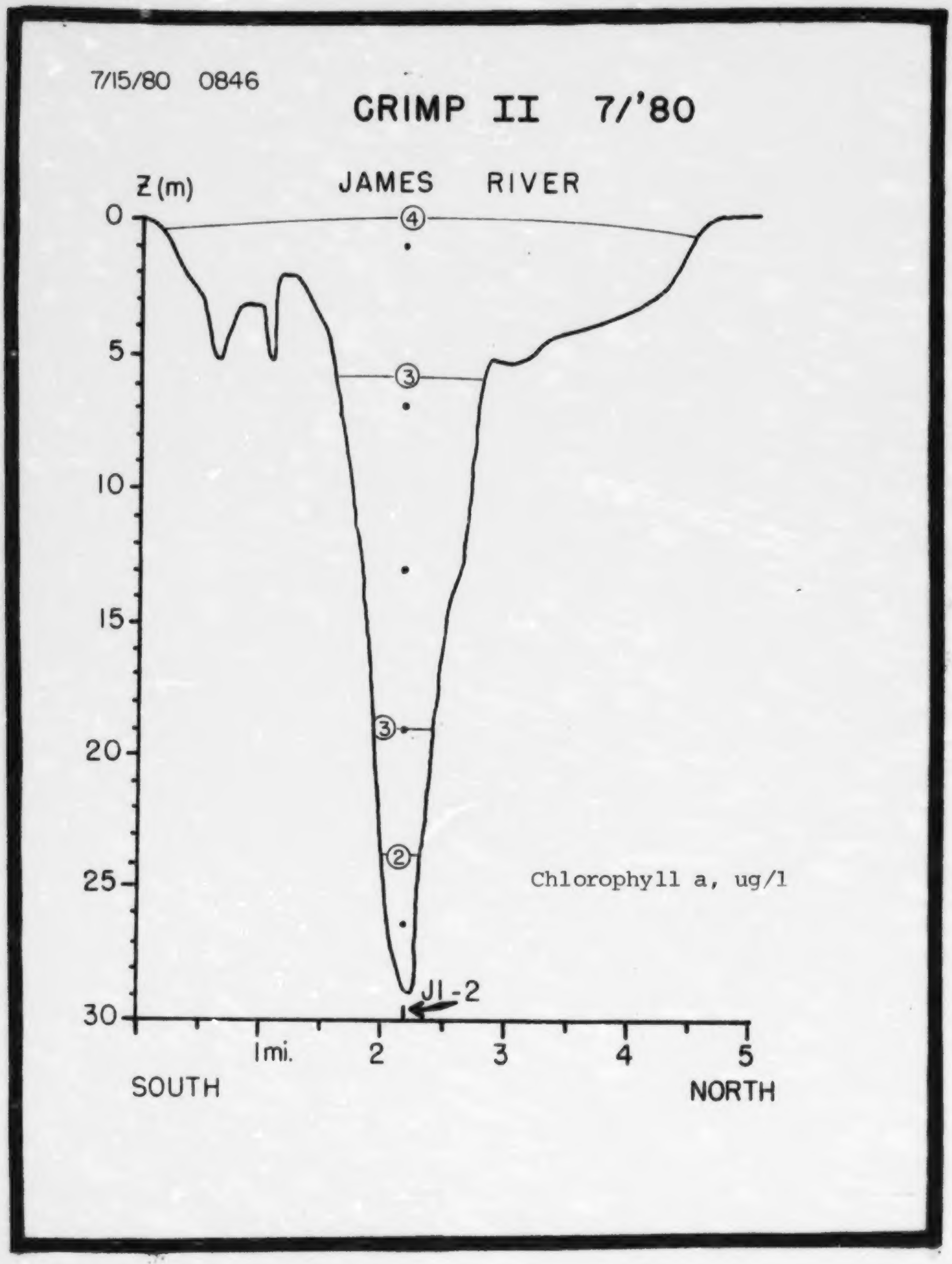
Chlorophyll a, ug/l

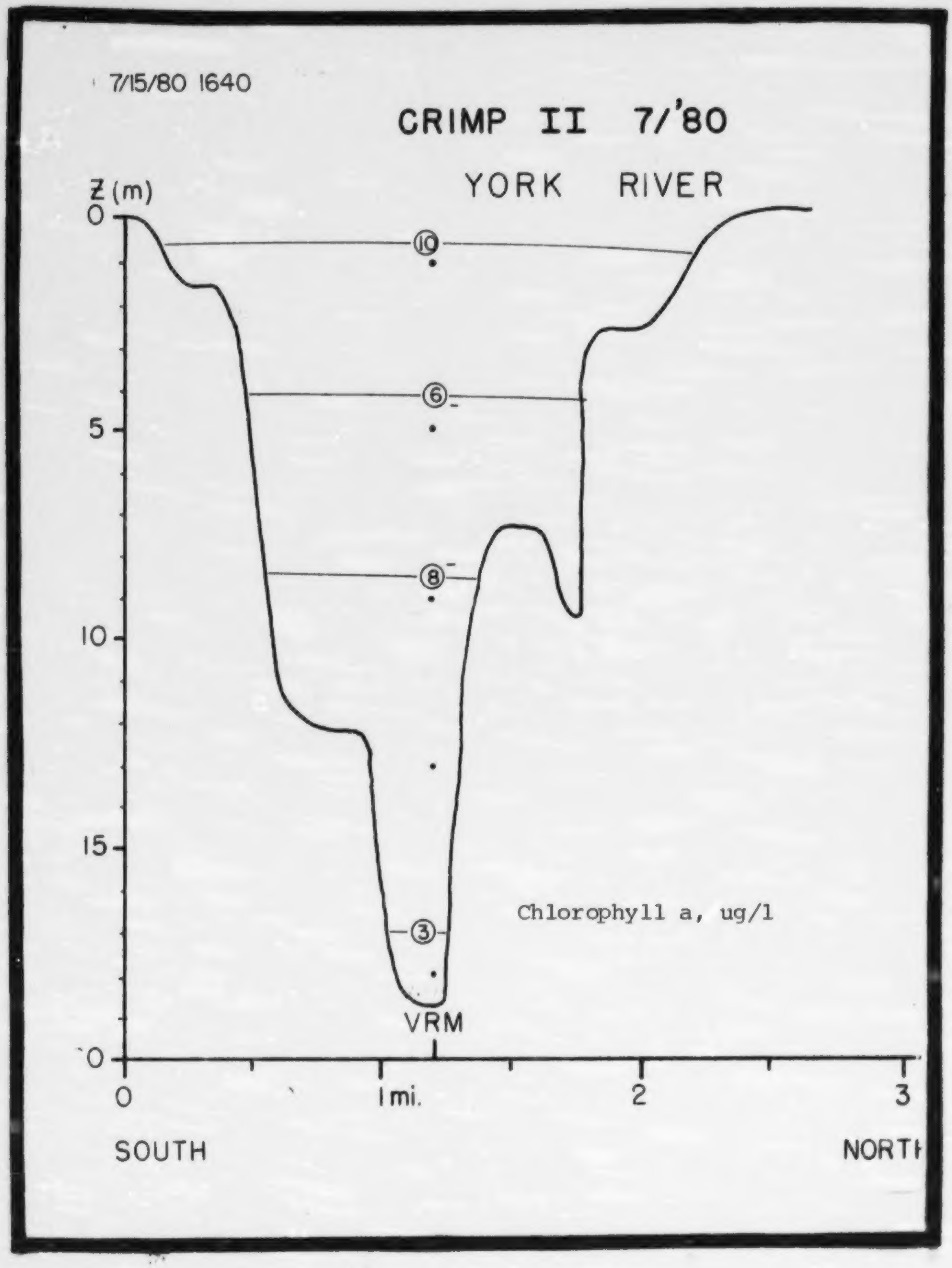

7/15/80 0733
CRIMP II 7/'80
WOLF TRAP
Z(m)
0
5
10
15
20
9
6
4
5
3
3
1
1
Chlorophyll a, ug/l
WT-1
WT-2
WT-3
WT-4
WT-5
0 mi.
5
10
15
WEST
EAST

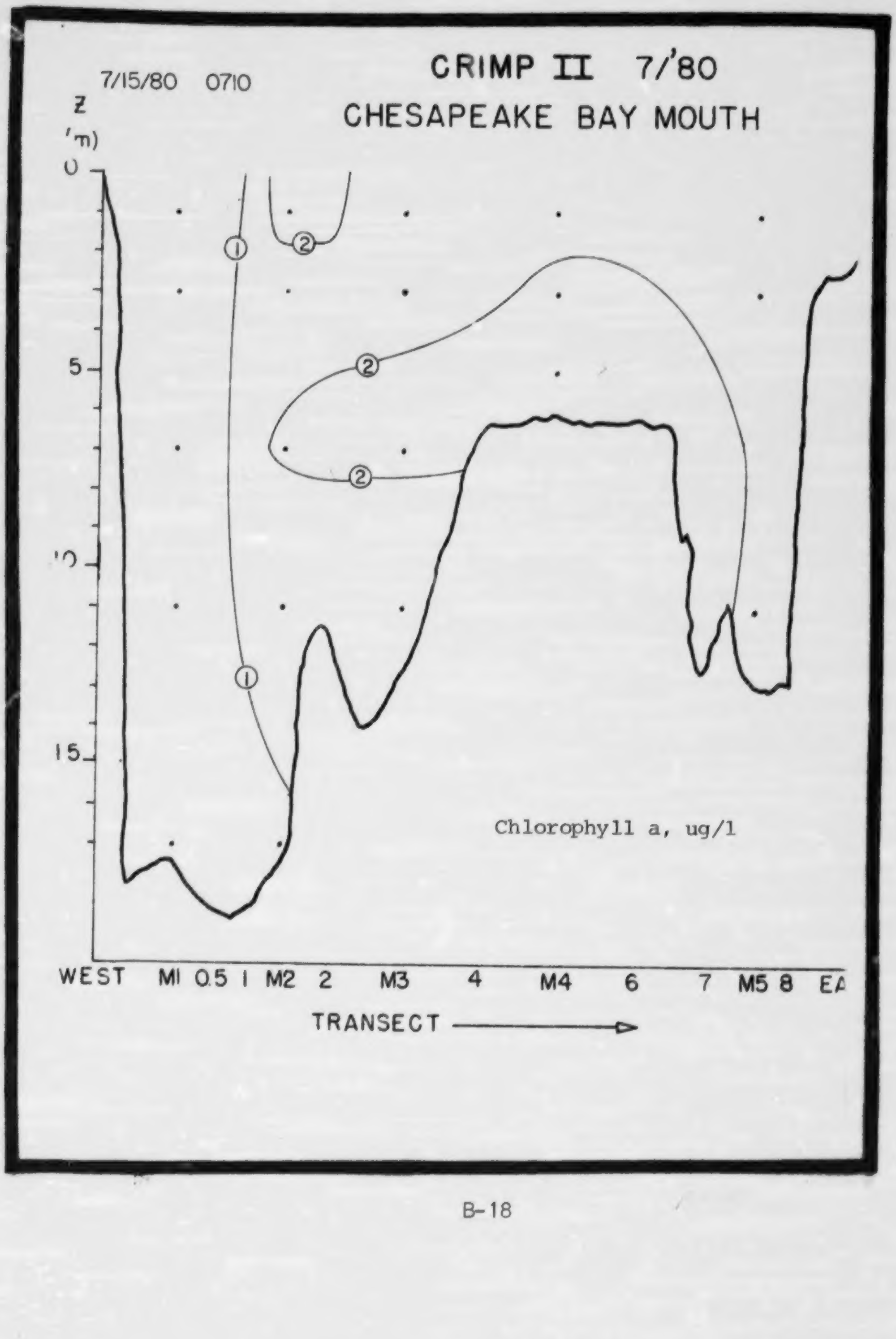
7/15/80 1235

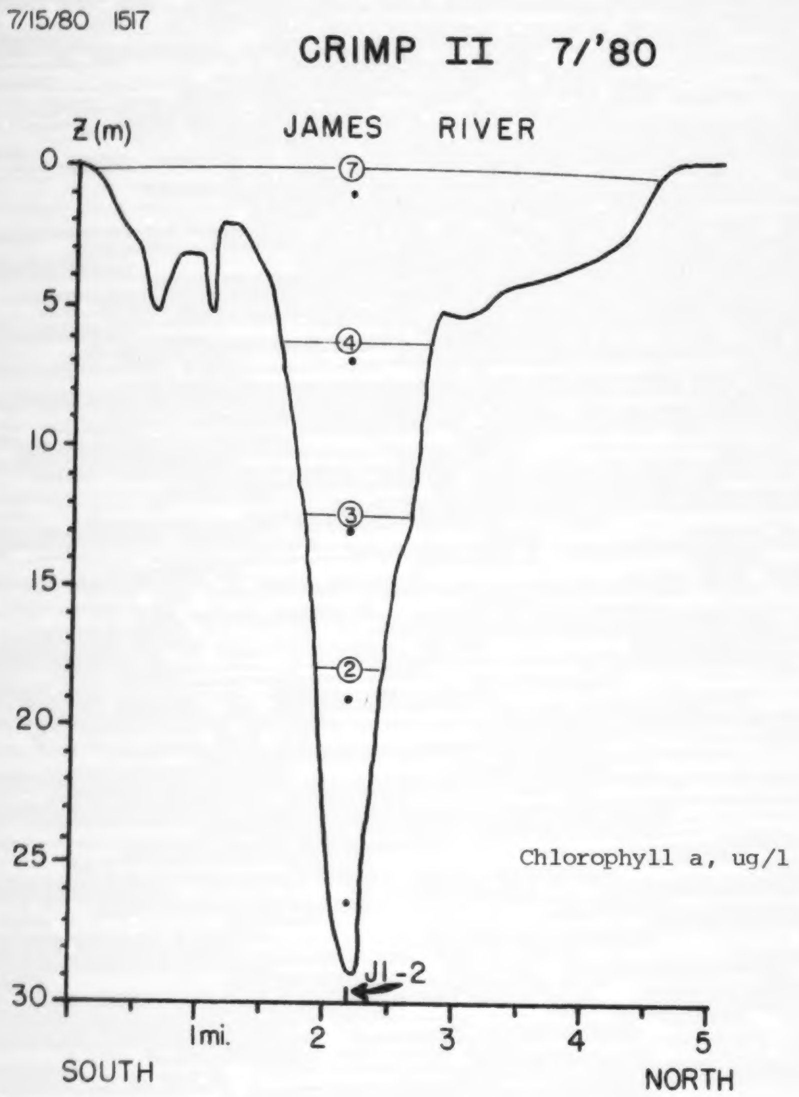

CRIMP II 7/'80
WOLF TRAP
Z(m)
0
5
10
15
20
8
8
7
5
7
9
5
3
1
1
Chlorophyll a, ug/l
WT-1
WT-2
WT-3
WT-4
WT-5
0 mi.
5
10
15
WEST
EAST

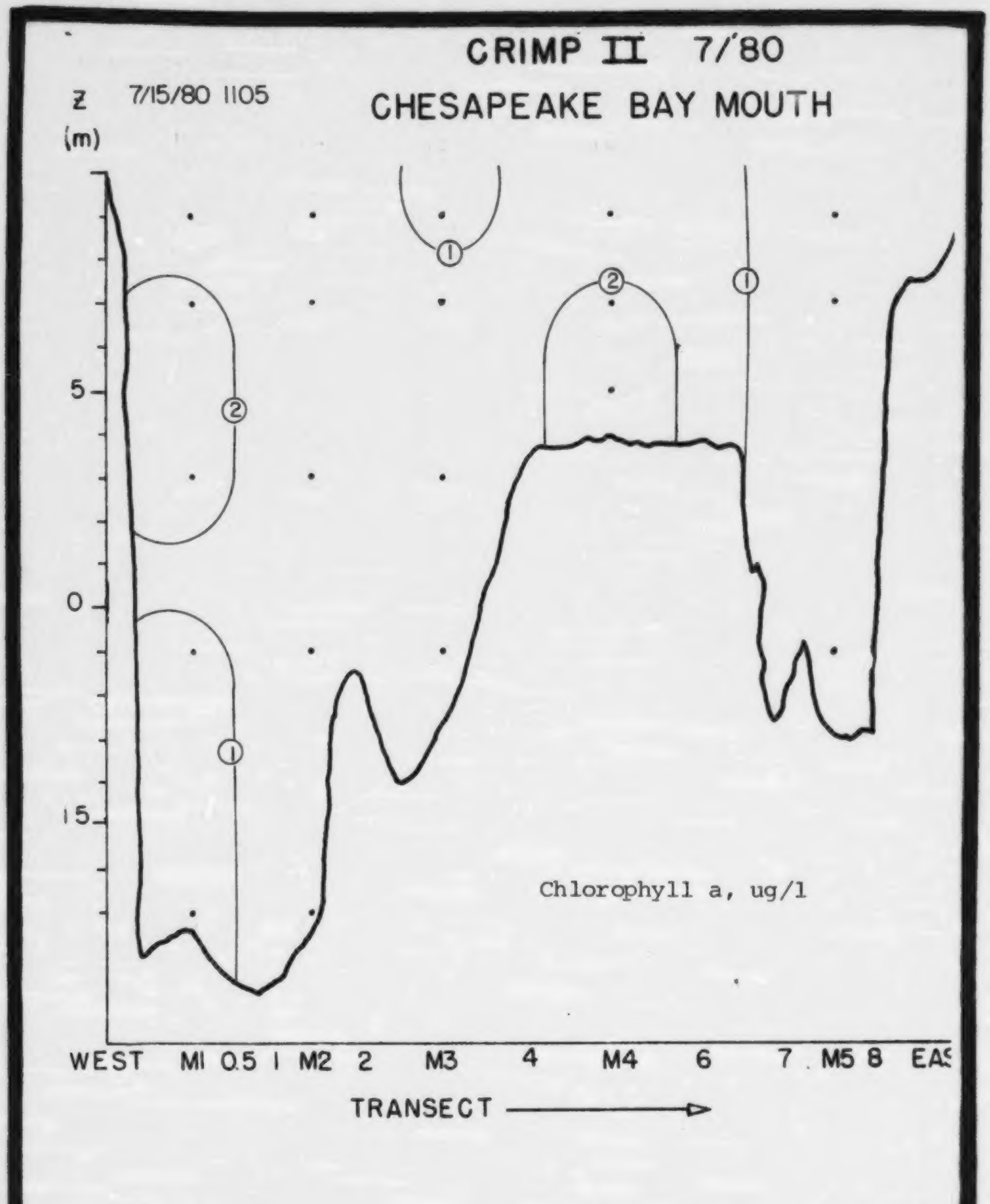

7/15/80 0618
CRIMP II 7/'80
YORK RIVER
Z (m)
0
5
10
15
0
⑪
⑦
②
①
VRM
Chlorophyll a, ug/l
0
1 mi.
2
3
SOUTH
NORT

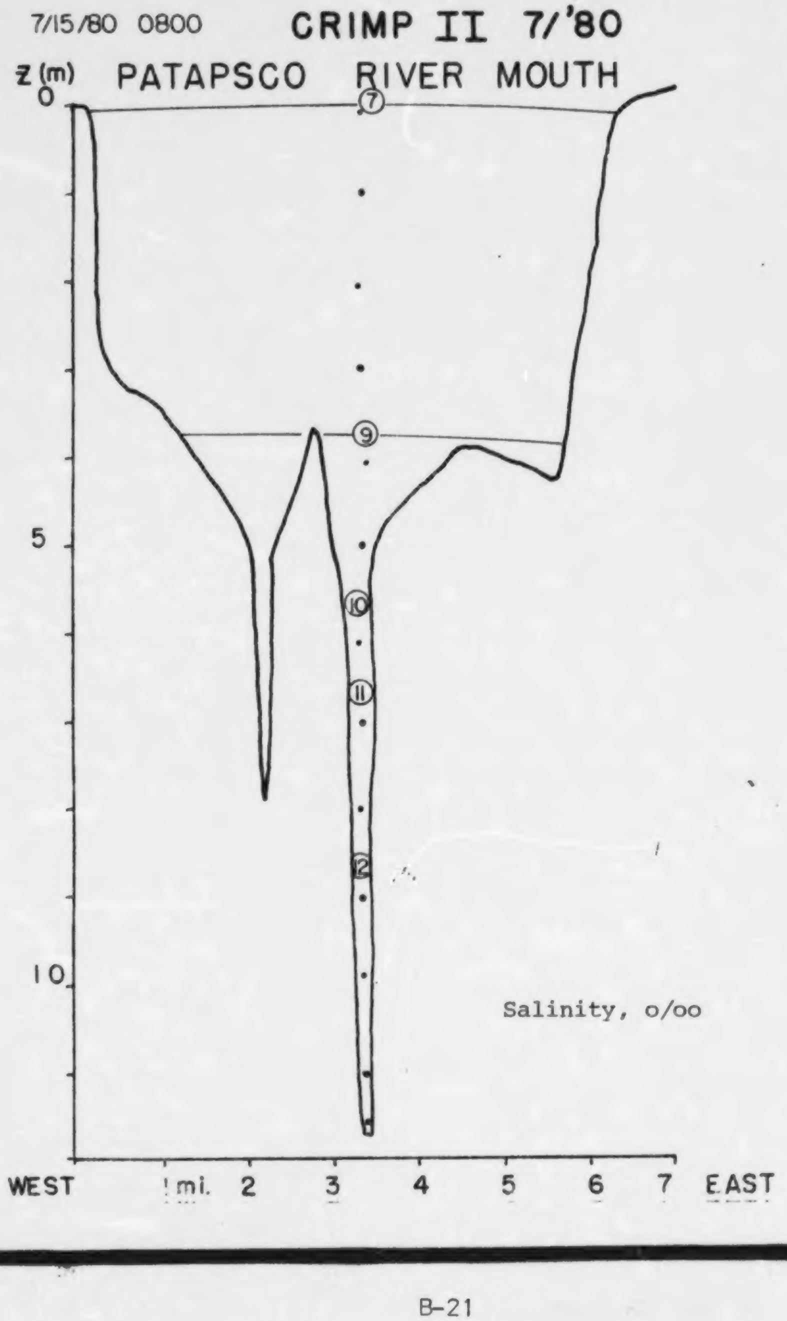

7/15/80 0846
CRIMP II 7/'80
JAMES RIVER
Z (m)
0
5
10
15
20
25
30
④
③
③
②
JI-2
Chlorophyll a, ug/l
1 mi.
2
3
4
5
SOUTH
NORTH


7/15/80 1640
CRIMP II 7/'80
YORK RIVER
Z (m)
0
5
10
15
0
⑩
⑥
⑧
③
Chlorophyll a, ug/l
VRM
0
1 mi.
2
3
SOUTH
NORT

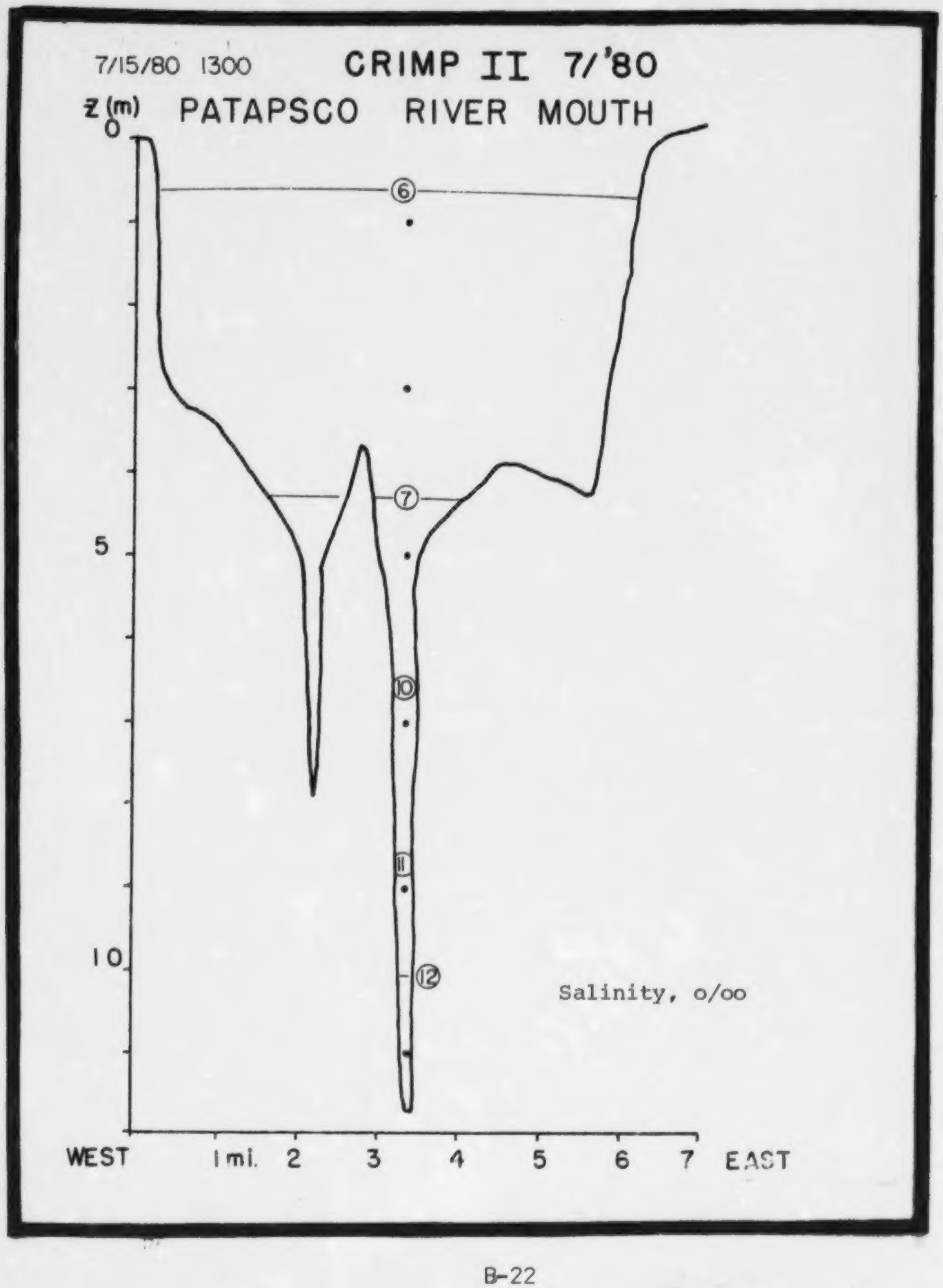

7/15/80 0710
CRIMP II 7/'80
CHESAPEAKE BAY MOUTH
Z
(m)
0
5
10
15
1
2
Chlorophyll a, ug/l
WEST
M1
0.5
1
M2
2
M3
4
M4
6
7
M5
8
EA
TRANSECT

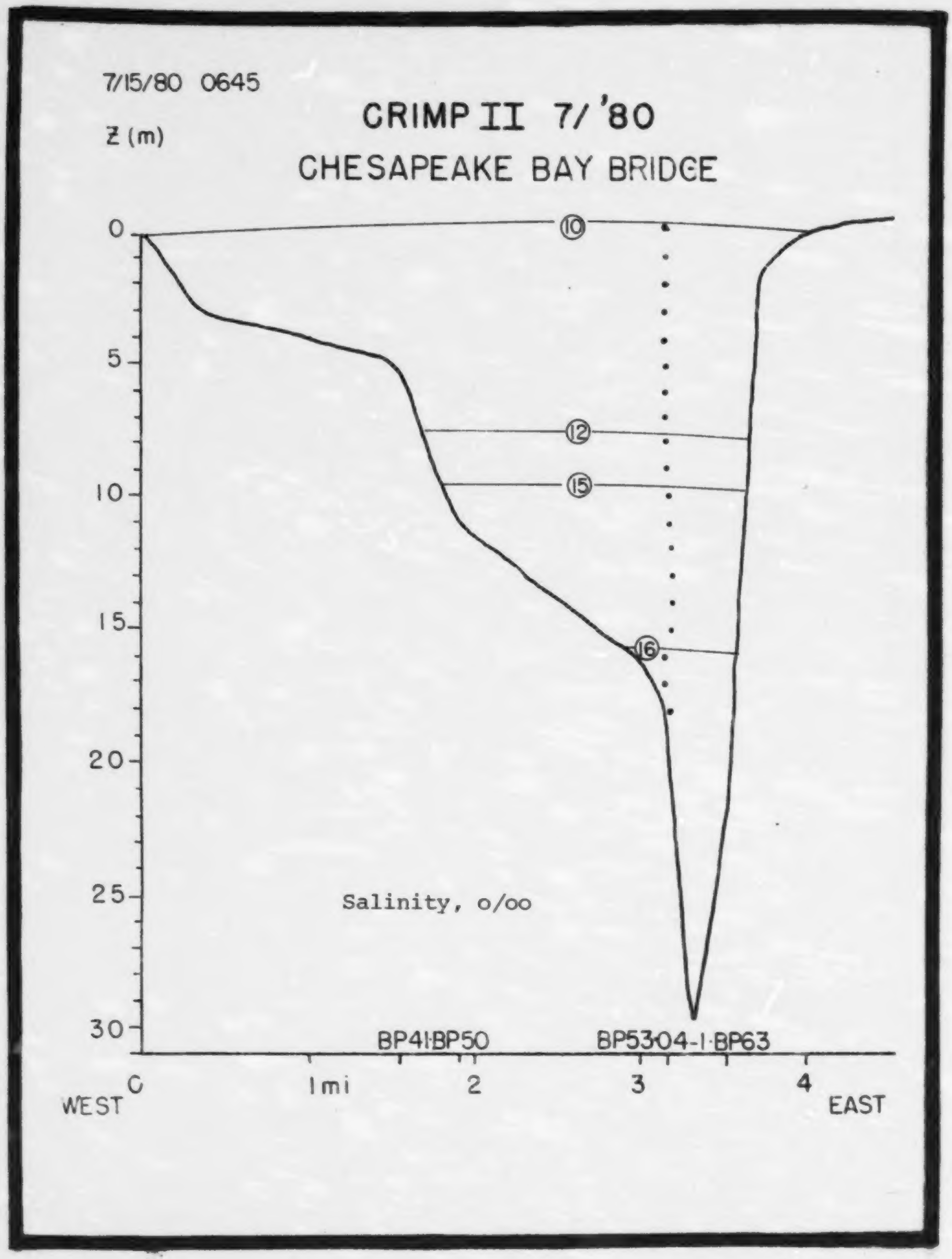

7/15/80 1517
CRIMP II 7/'80
Z(m)
JAMES RIVER
0
5
10
15
20
25
30
⑦
④
③
②
Chlorophyll a, ug/l
J1-2
1mi.
2
3
4
5
SOUTH
NORTH

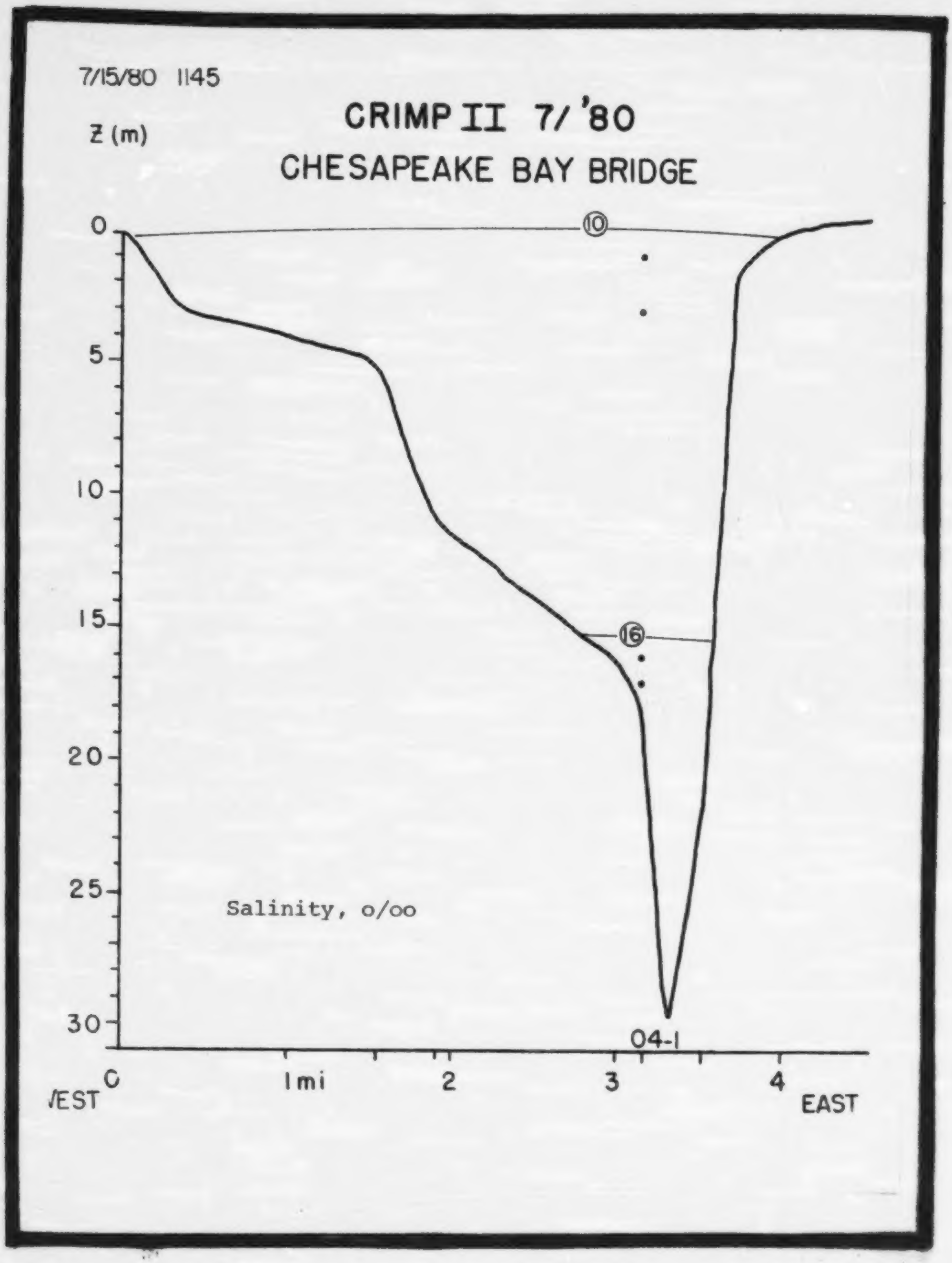
CRIMP II 7/'80
CHESAPEAKE BAY MOUTH
z
(m)
7/15/80 1105
5
0
15
①
②
②
①
①
Chlorophyll a, ug/l
WEST
M1
0.5
1
M2
2
M3
4
M4
6
7
M5
8
EAS
TRANSECT

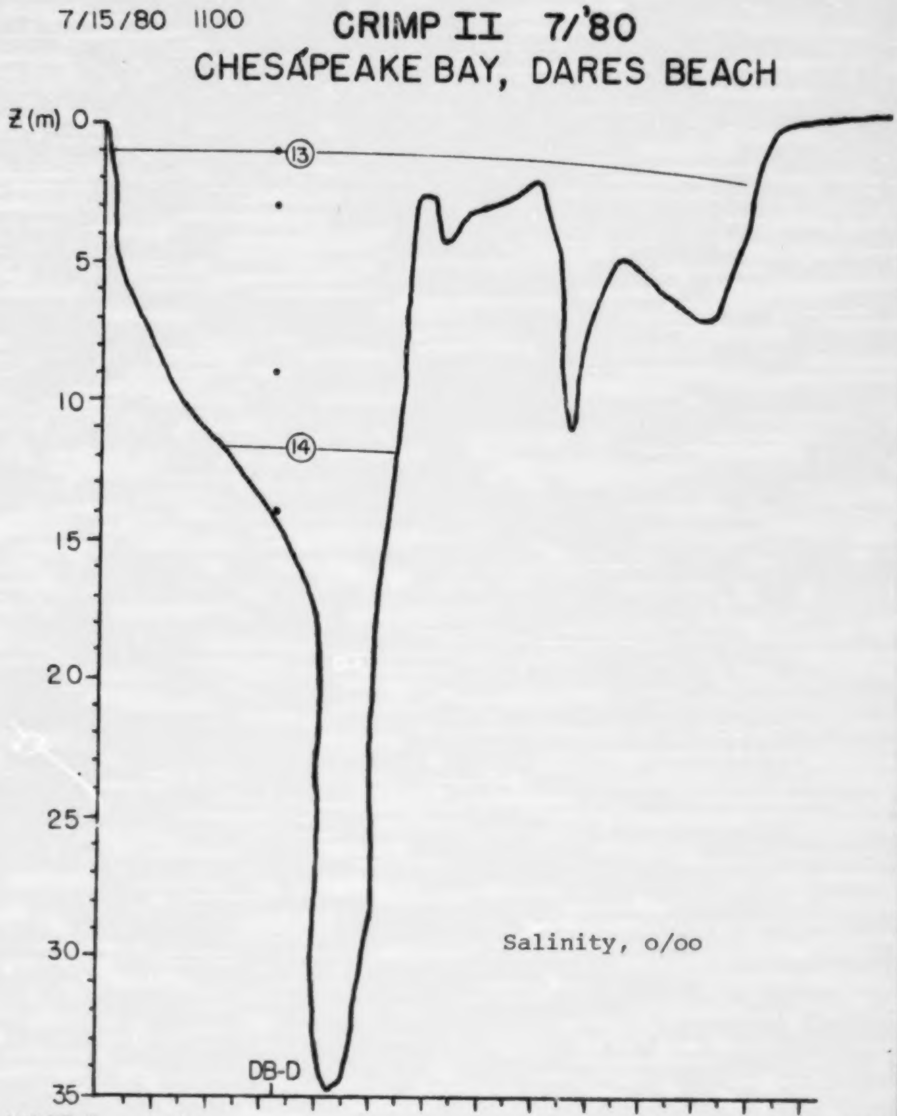


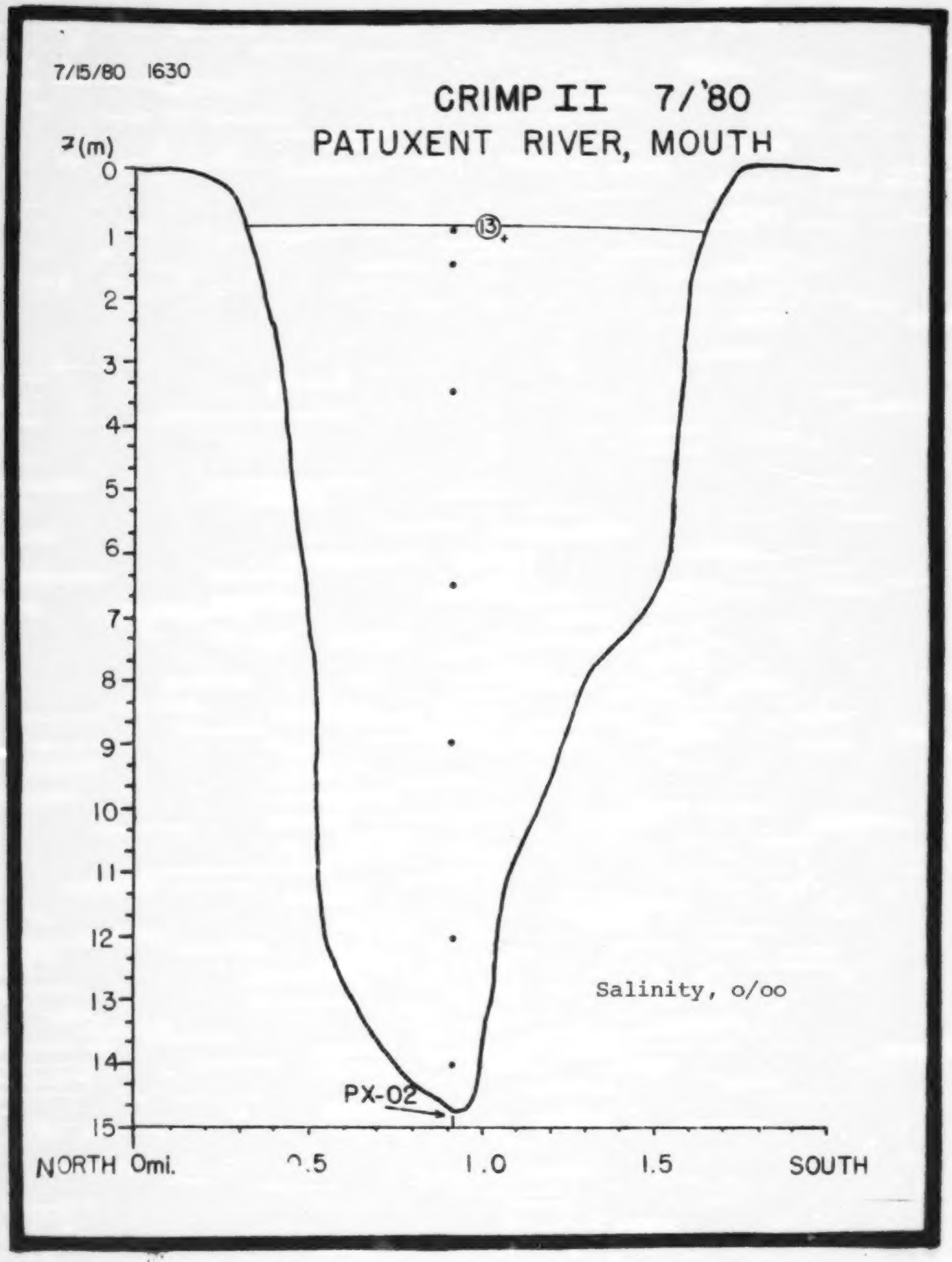
7/15/80 0800
CRIMP II 7/'80
z (m)
PATAPSCO RIVER MOUTH
0
⑦
⑨
5
⑩
⑪
⑫
10
Salinity, o/oo
WEST
1 mi.
2
3
4
5
6
7
EAST


7/15/80 1300
CRIMP II 7/'80
PATAPSCO RIVER MOUTH
z (m)
0
5
10
6
7
10
11
12
Salinity, o/oo
WEST
1 mi.
2
3
4
5
6
7
EAST


7/15/80 0645
CRIMP II 7/'80
CHESAPEAKE BAY BRIDGE
Z (m)
0
5
10
15
20
25
30
⑩
⑫
⑮
⑯
Salinity, o/oo
BP41·BP50
BP53·04-1·BP63
0
1mi
2
3
4
WEST
EAST


7/15/80 1145
CRIMP II 7/'80
CHESAPEAKE BAY BRIDGE
Z (m)
0
5
10
15
20
25
30
⑩
⑯
04-1
Salinity, o/oo
0
1mi
2
3
4
/EST
EAST

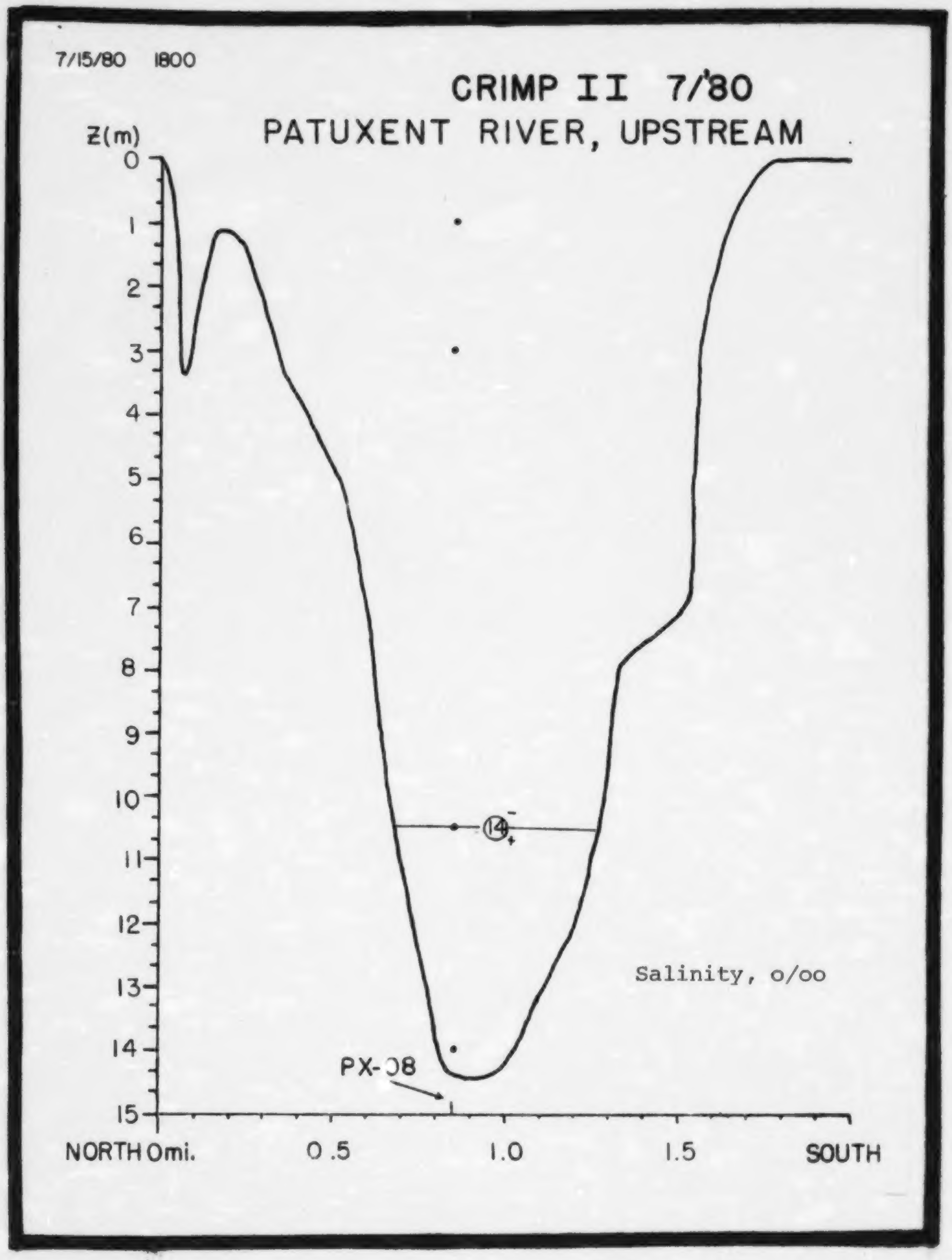

7/15/80 1100
CRIMP II 7/'80
CHESAPEAKE BAY, DARES BEACH
Z(m) 0
5
10
15
20
25
30
35
13
14
DB-D
WEST 0
1 mi.
5
10
13 EAST
Salinity, o/oo

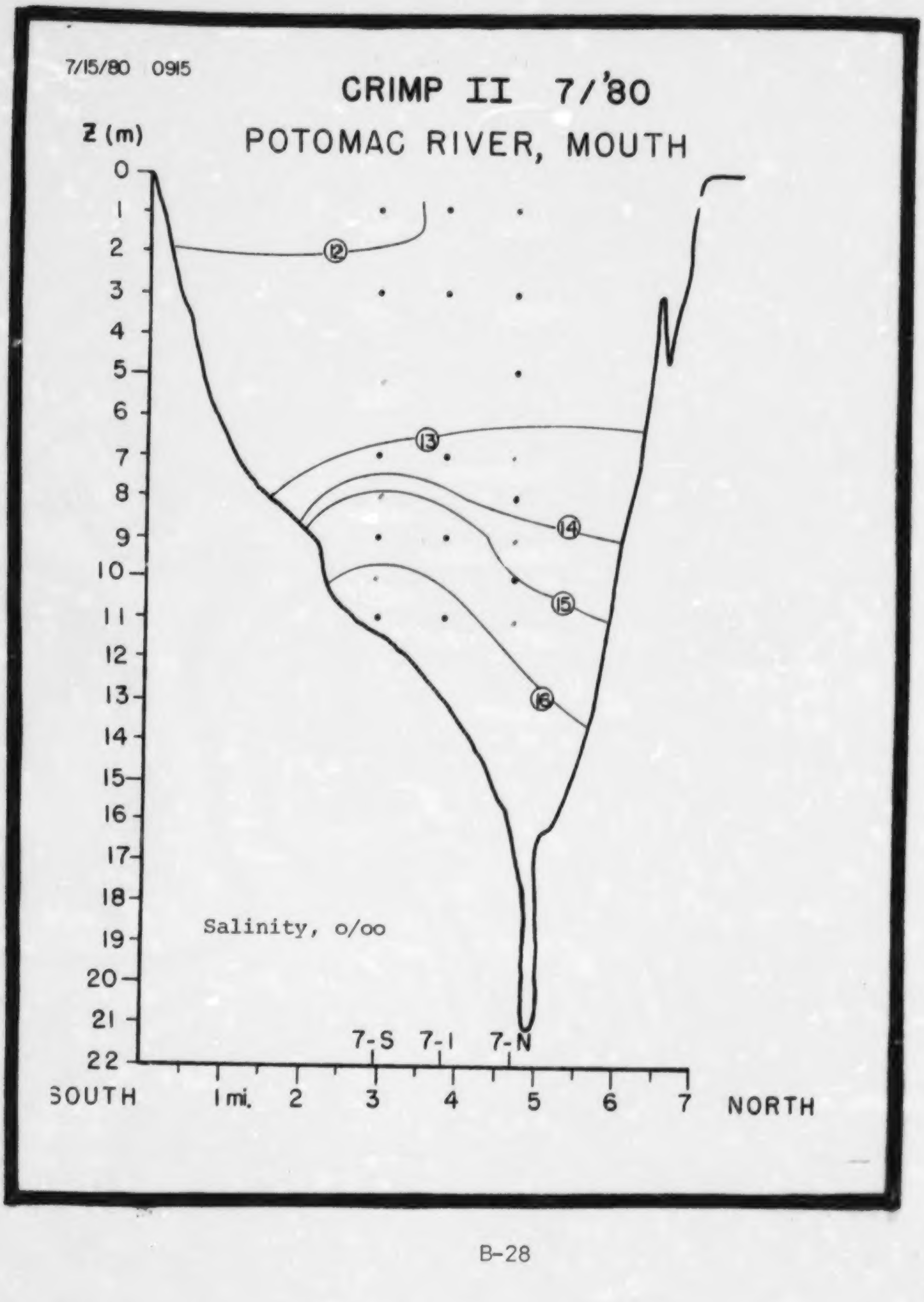


7/15/80 1630

CRIMP II 7/'80

PATUXENT RIVER, MOUTH


7/15/80 1800
CRIMP II 7/80
PATUXENT RIVER, UPSTREAM
Z(m)
0
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
14
Salinity, o/oo
PX-08
NORTH 0mi.
0.5
1.0
1.5
SOUTH

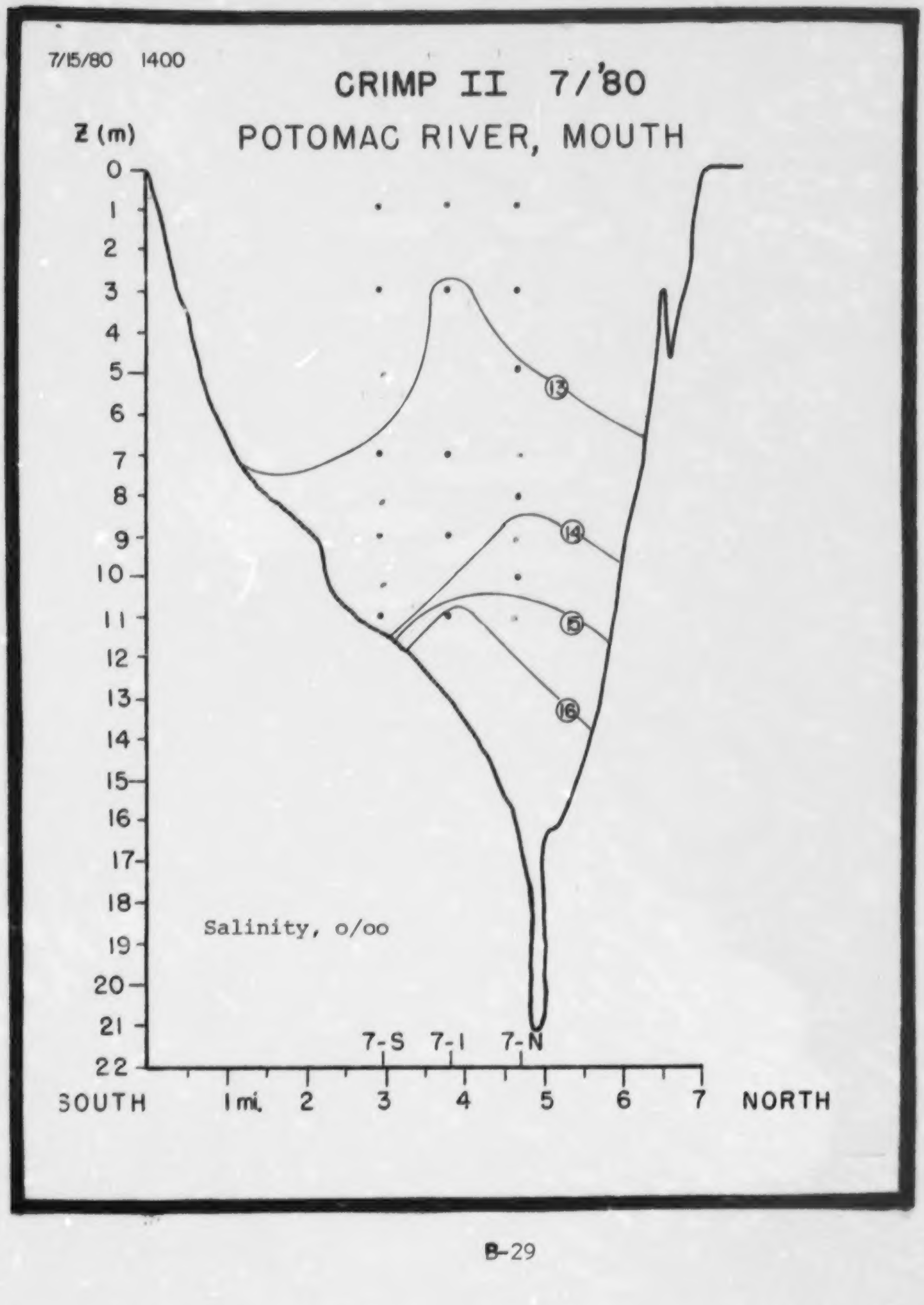


7/15/80 0915

CRIMP II 7/'80

POTOMAC RIVER, MOUTH

Salinity, o/oo

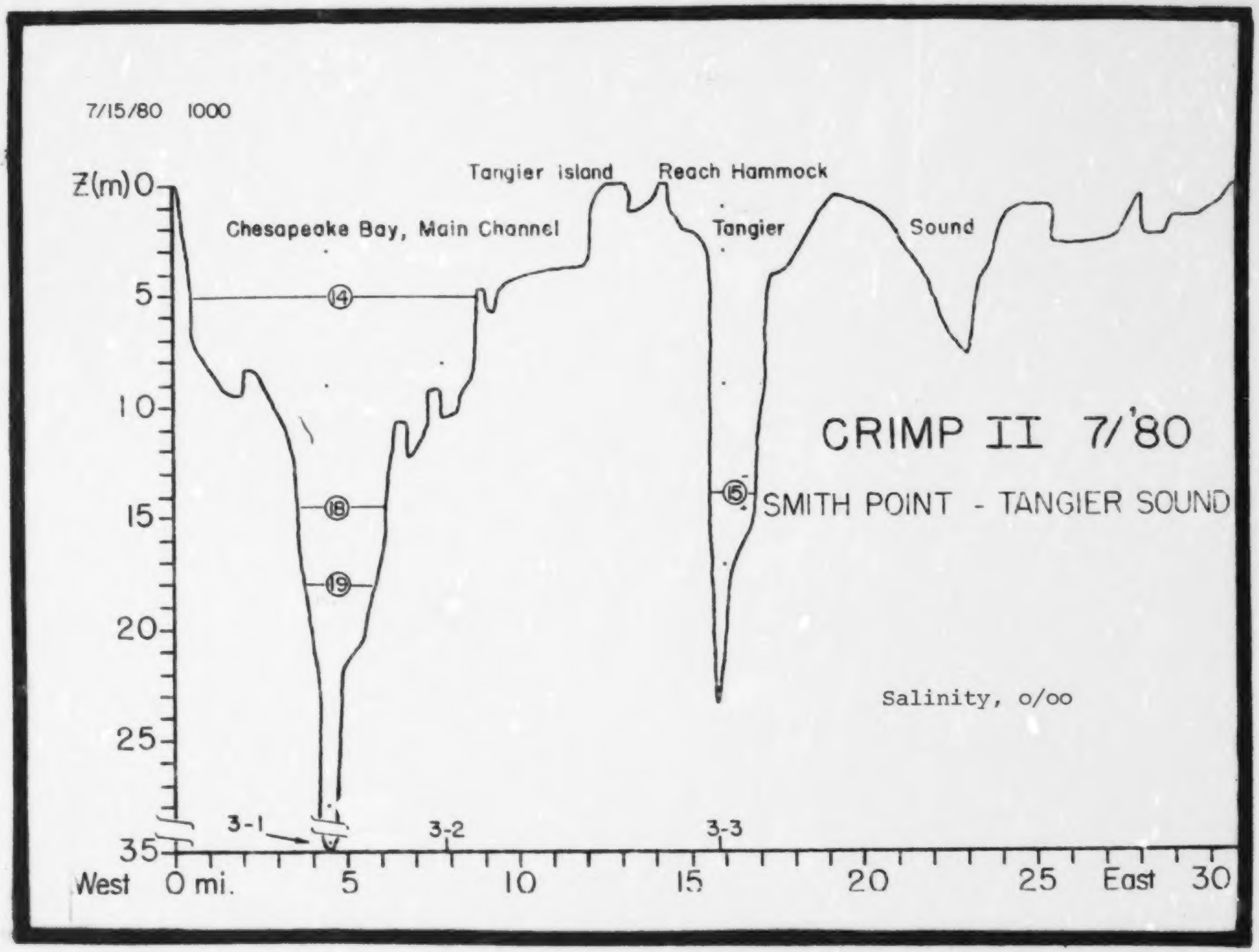


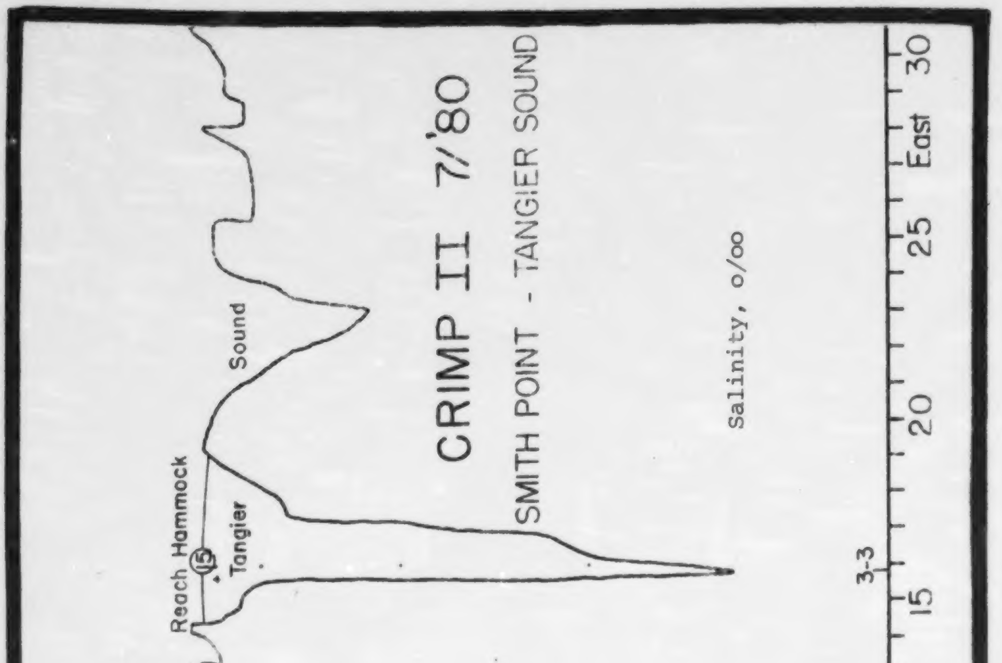
7/15/80 1400

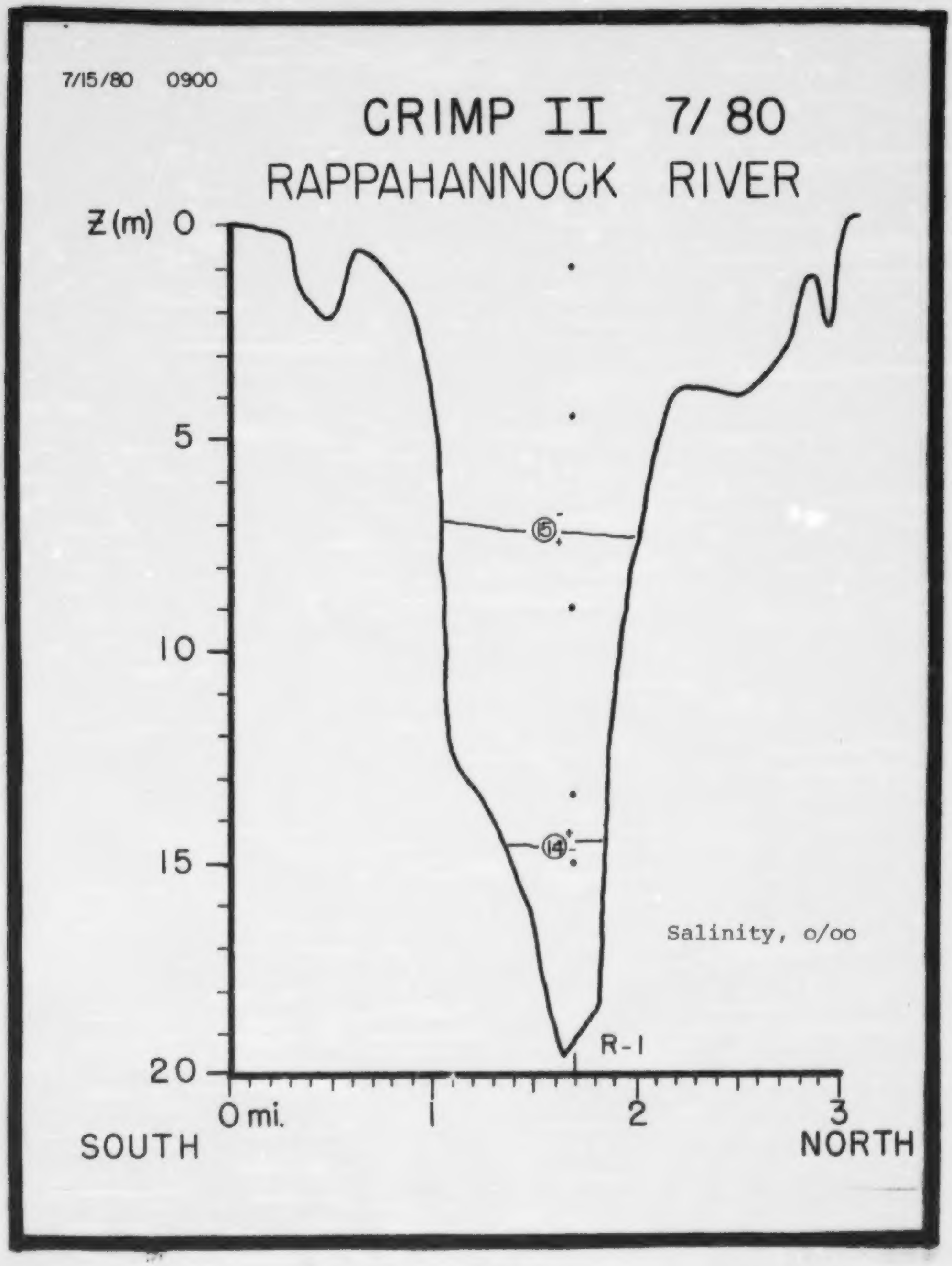
CRIMP II 7/'80

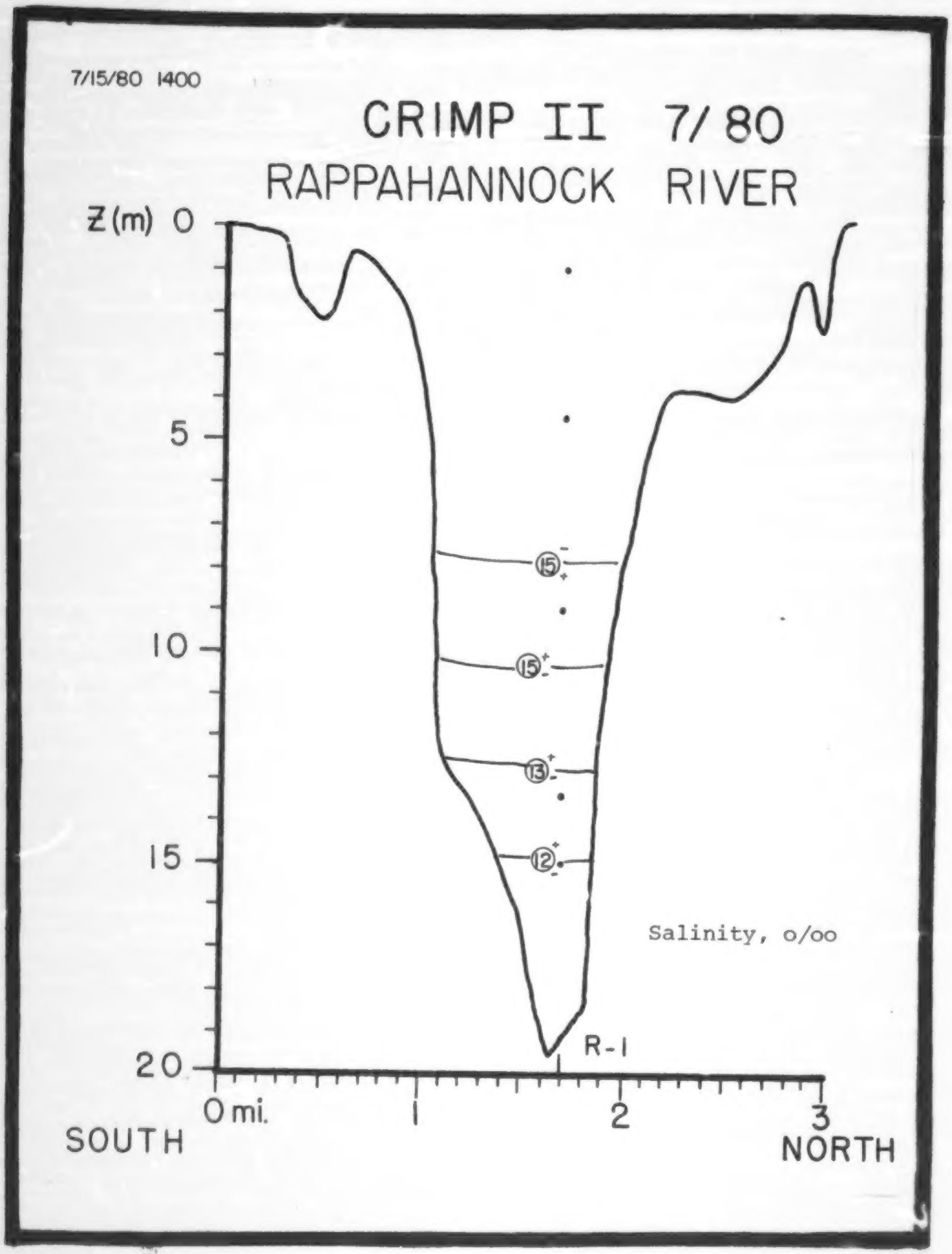
POTOMAC RIVER, MOUTH

Salinity, o/oo


7/15/80 1000
CRIMP II 7/'80
SMITH POINT - TANGIER SOUND
Salinity, o/oo
Z(m)
0
5
10
15
20
25
35
West
0 mi.
5
10
15
20
25
East
30
3-1
3-2
3-3
Chesapeake Bay, Main Channel
Tangier Island
Reach Hammock
Tangier
Sound
⑭
⑱
⑲
⑮


7/15/80 1610
Z(m) 0
5
10
15
20
25
35
West
0 mi.
5
10
15
20
25
30
East
3-1
3-2
3-3
Chesapeake Bay, Main Channel
Tangier Island
Reach Hammock
Tangier
Sound
14
15
16
19
15
CRIMP II 7/'80
SMITH POINT - TANGIER SOUND
Salinity, o/oo


7/15/80 0900
CRIMP II 7/80
RAPPAHANNOCK RIVER
Z(m) 0
5
10
15
20
⑮
⑭
Salinity, o/oo
R-1
0 mi.
1
2
3
SOUTH
NORTH

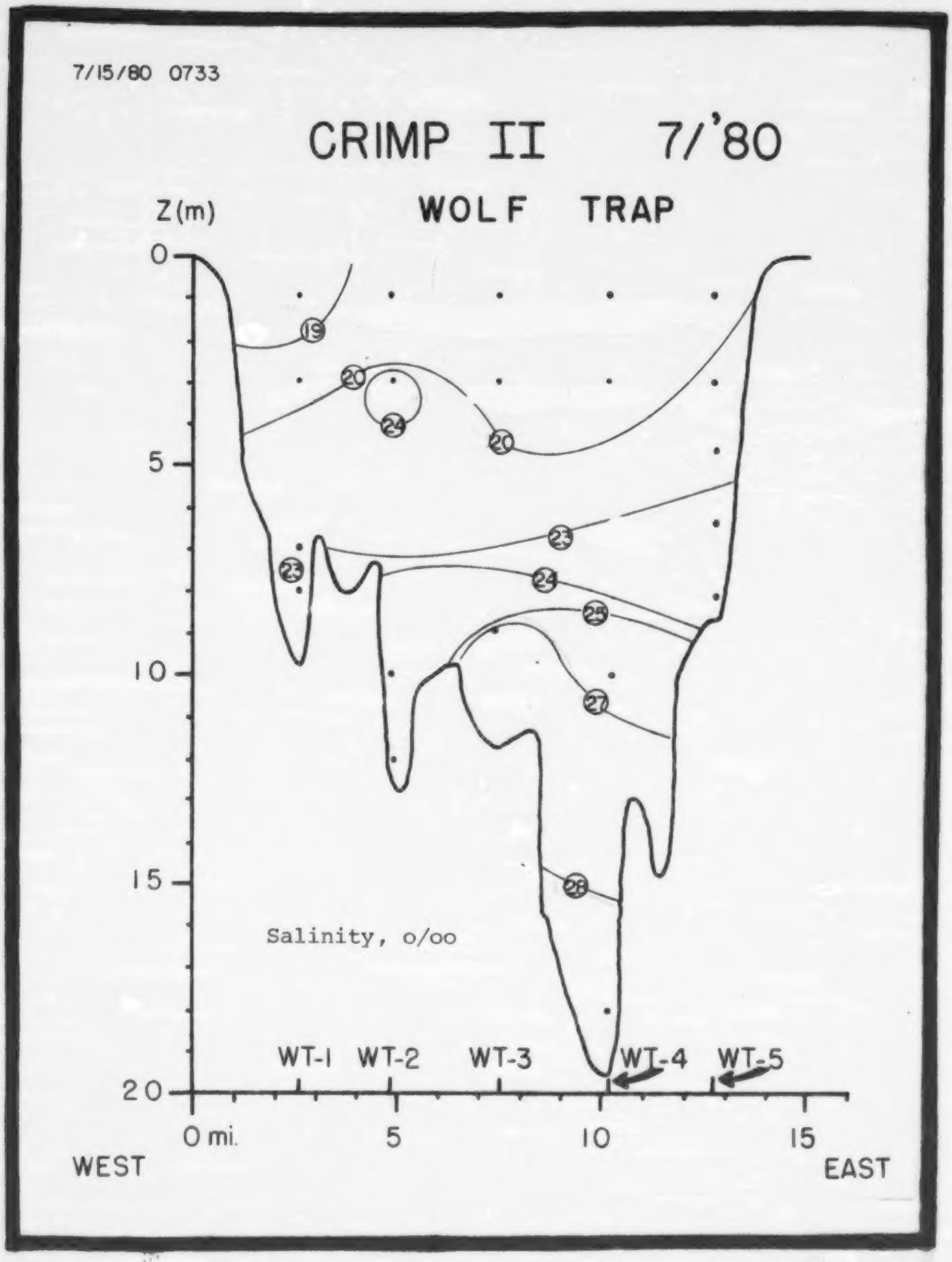


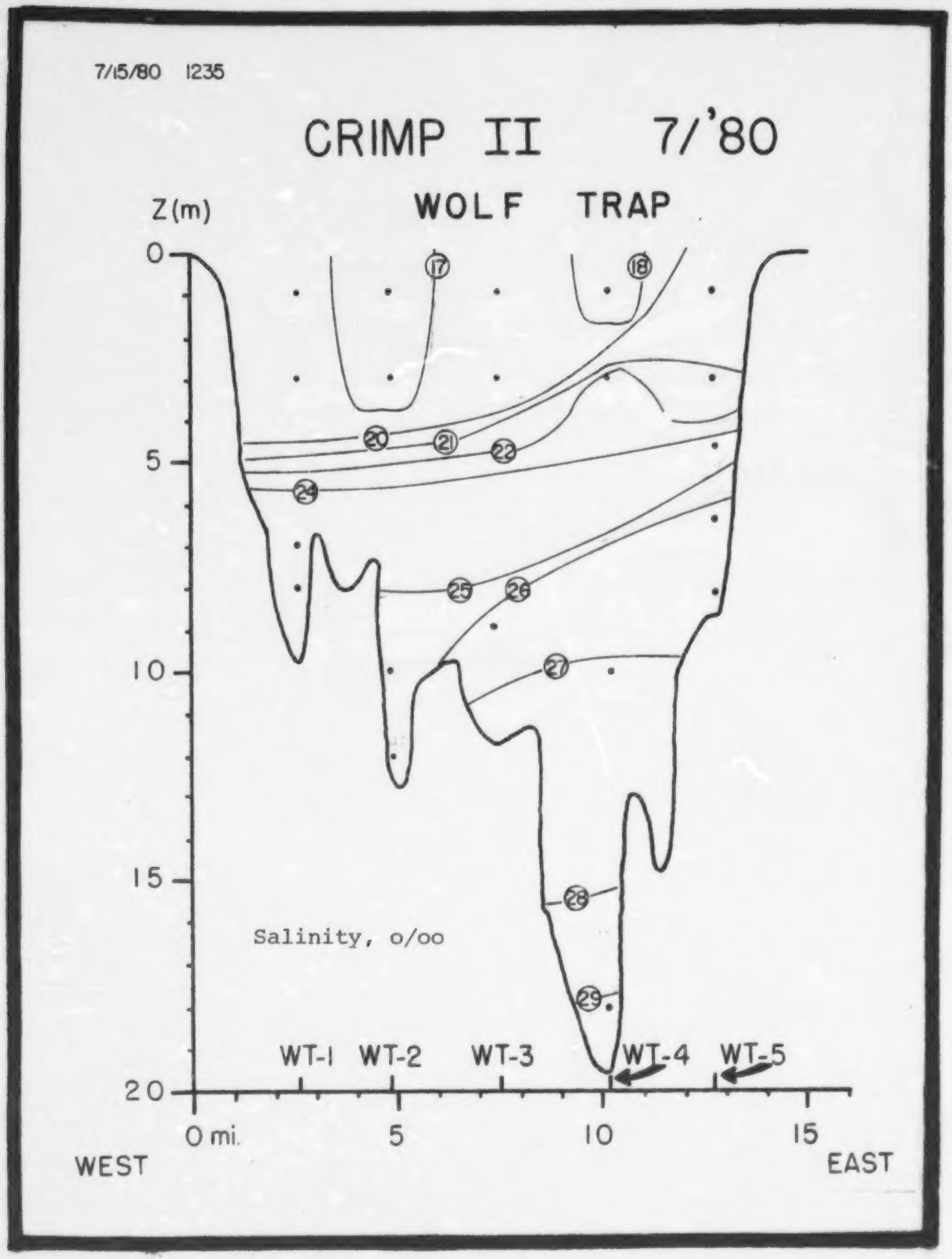
7/15/80 1400

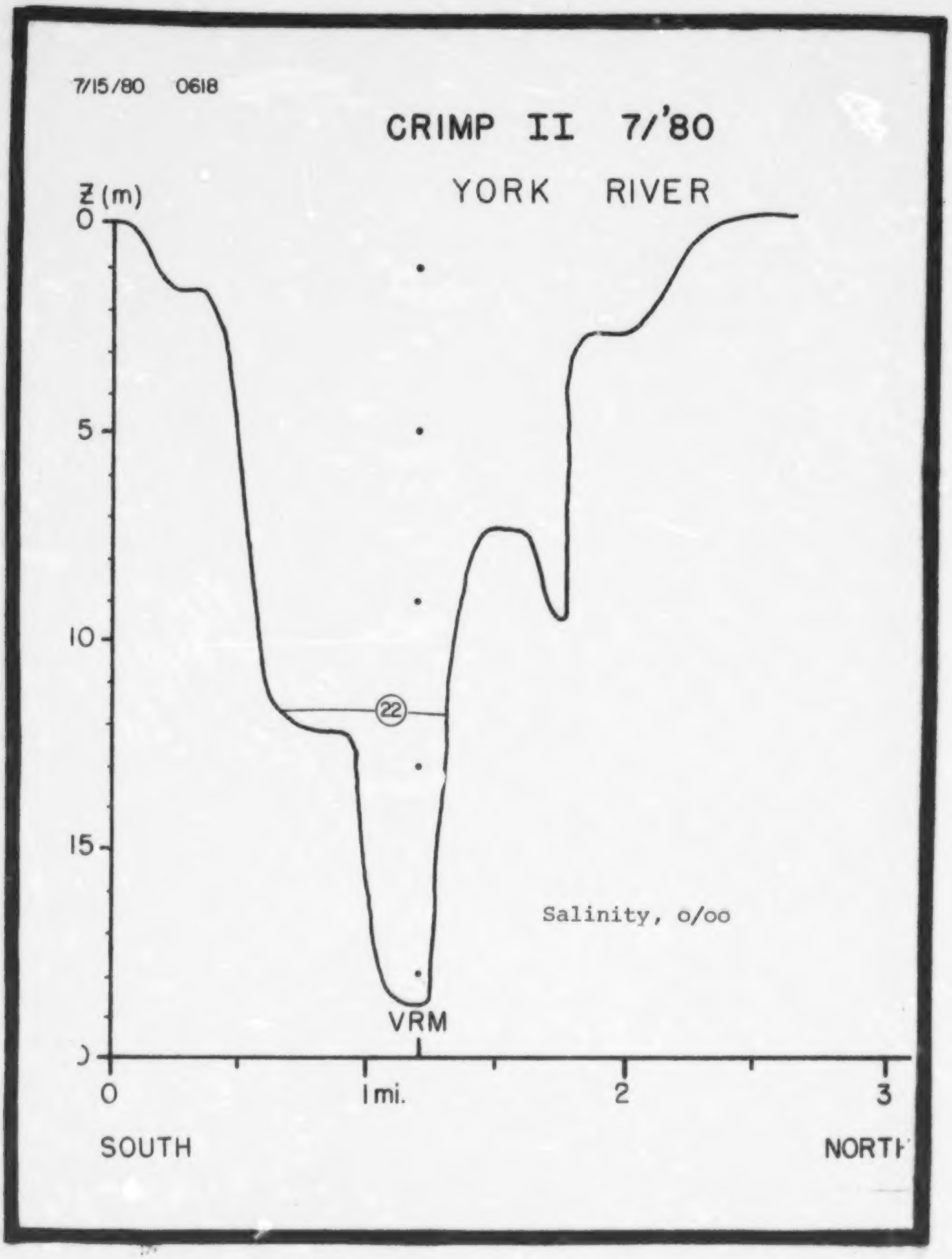
CRIMP II 7/80

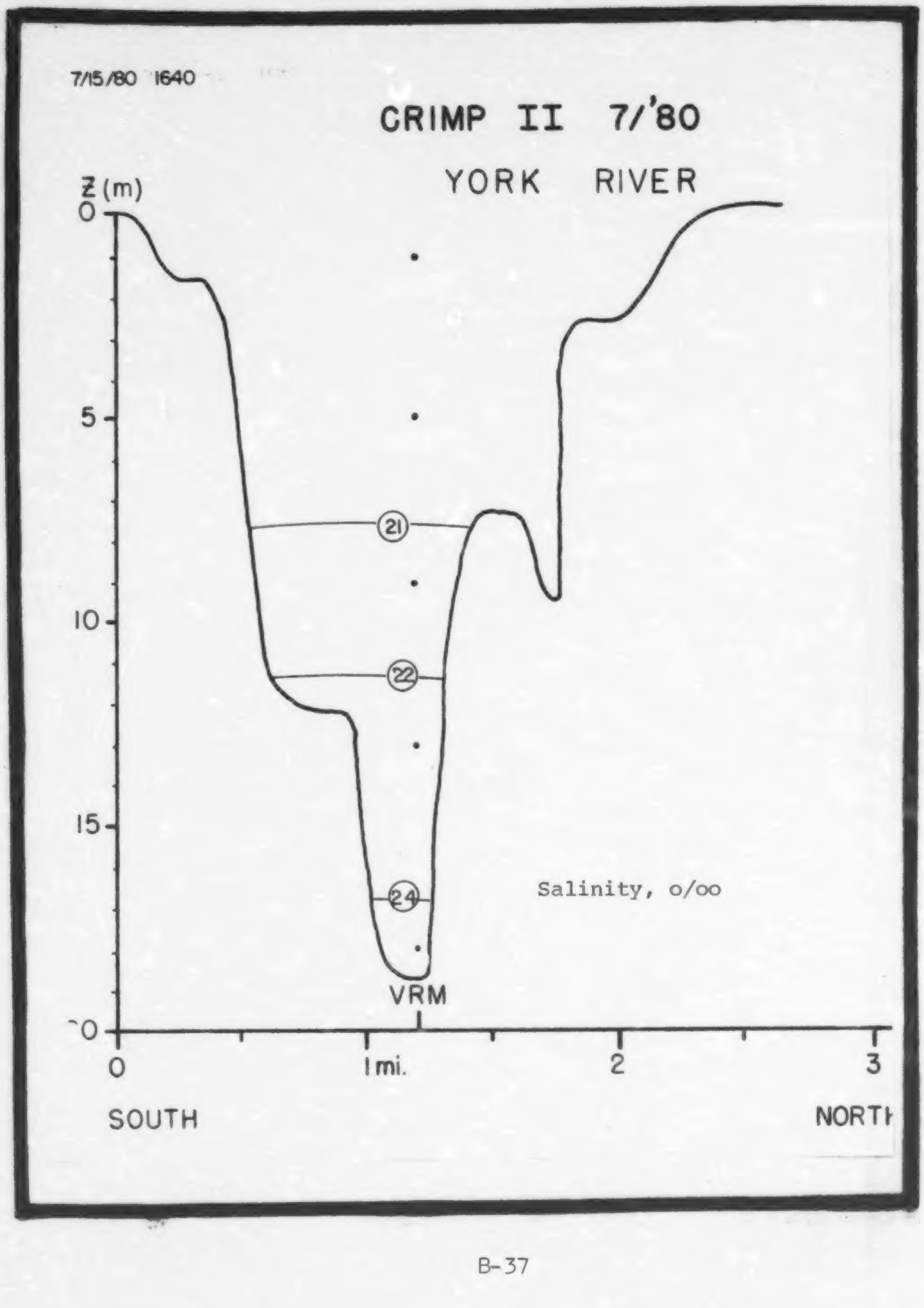
RAPPAHANNOCK RIVER

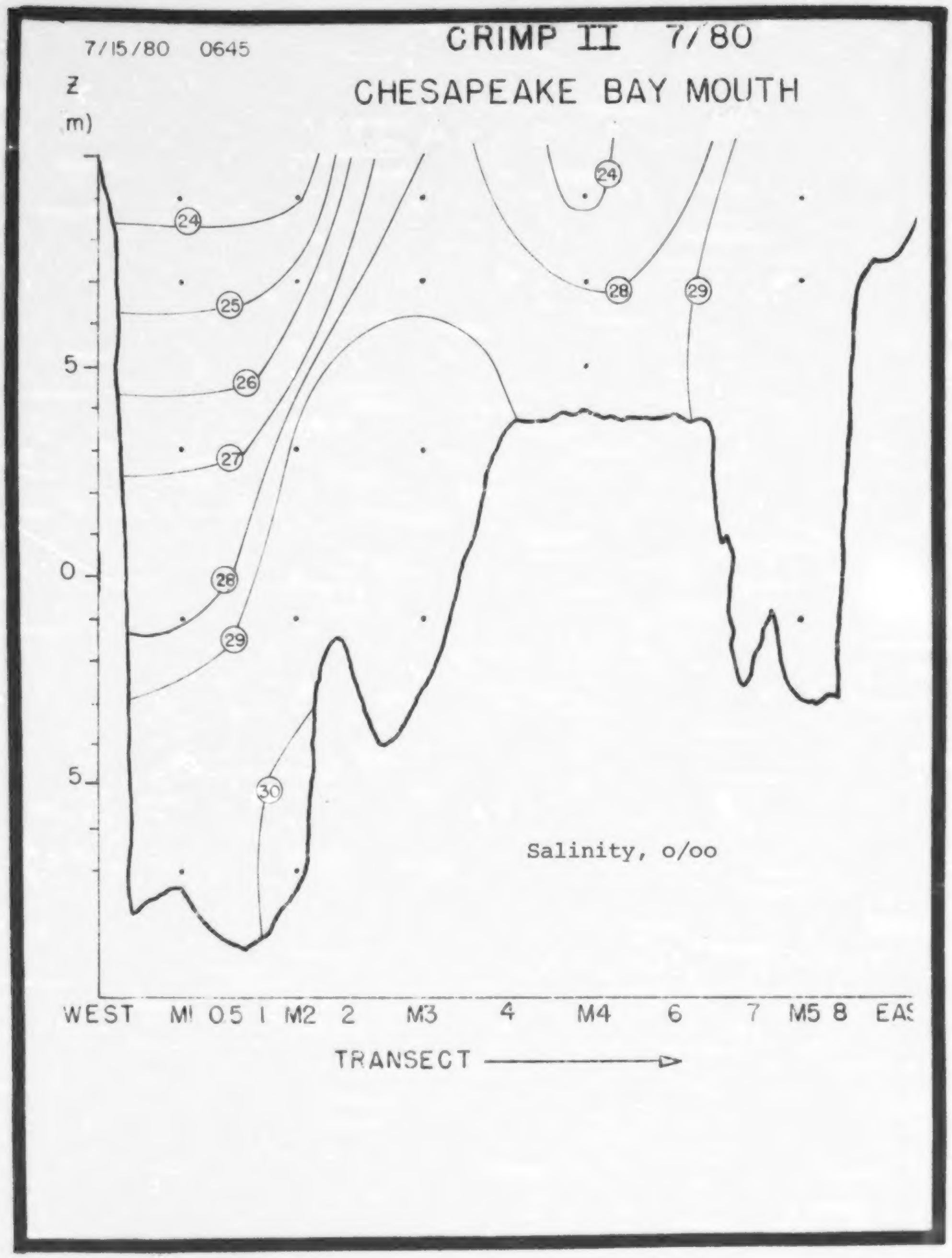
Salinity, o/oo

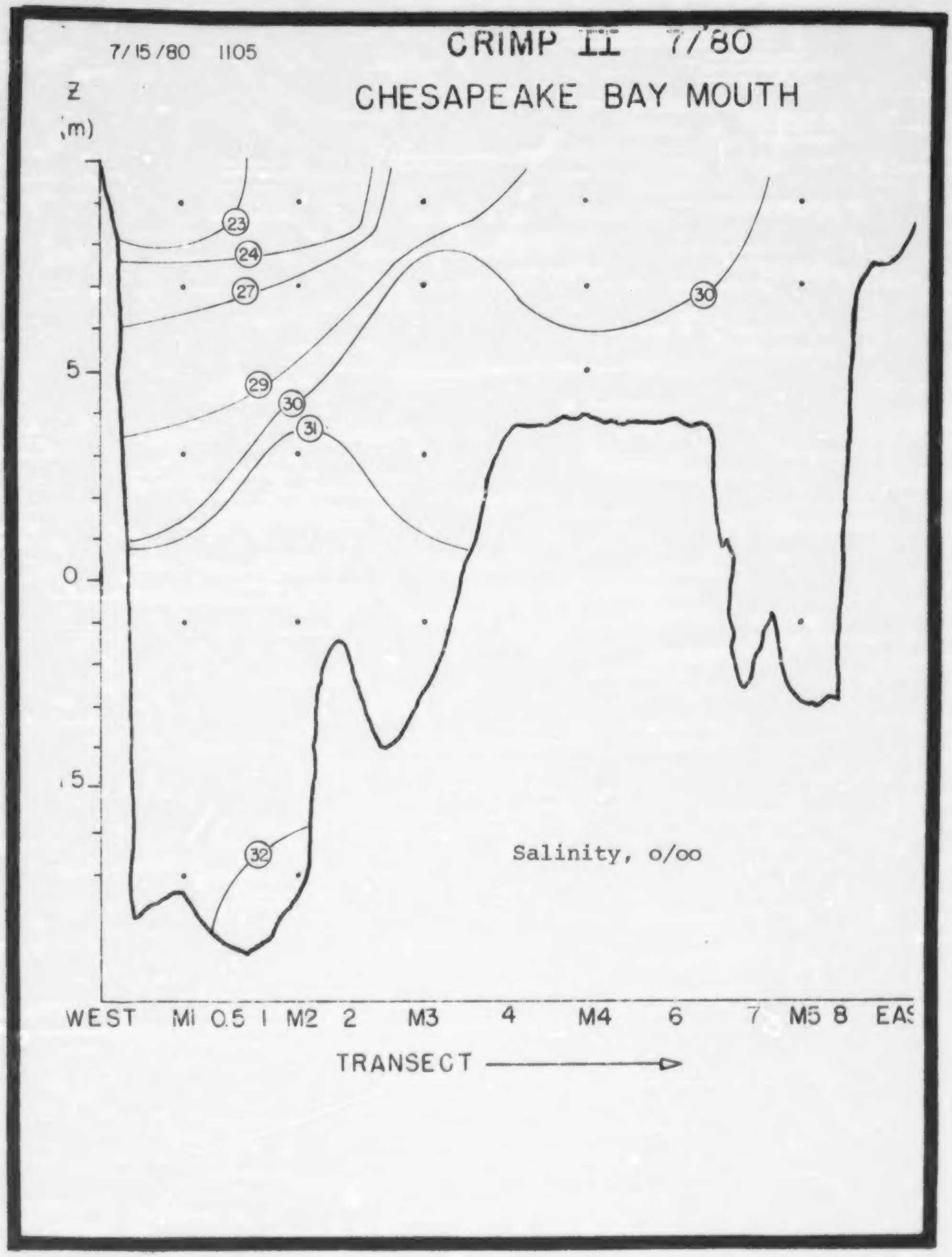

7/15/80 0733
CRIMP II 7/'80
WOLF TRAP
Z(m)
0
5
10
15
20
19
20
24
20
23
23
24
25
27
28
Salinity, o/oo
WT-1
WT-2
WT-3
WT-4
WT-5
0 mi.
5
10
15
WEST
EAST


7/15/80 1235
CRIMP II 7/'80
WOLF TRAP
Z(m)
0
5
10
15
20
17
18
20
21
22
24
25
26
27
28
29
Salinity, o/oo
WT-1
WT-2
WT-3
WT-4
WT-5
0 mi.
5
10
15
WEST
EAST


7/15/80 0618
CRIMP II 7/'80
YORK RIVER
Z (m)
0
5
10
15
22
Salinity, o/oo
VRM
0
1 mi.
2
3
SOUTH
NORTH


7/15/80 1640
CRIMP II 7/'80
YORK RIVER
Z (m)
0
5
10
15
~0
21
22
24
Salinity, o/oo
VRM
0
1 mi.
2
3
SOUTH
NORTH

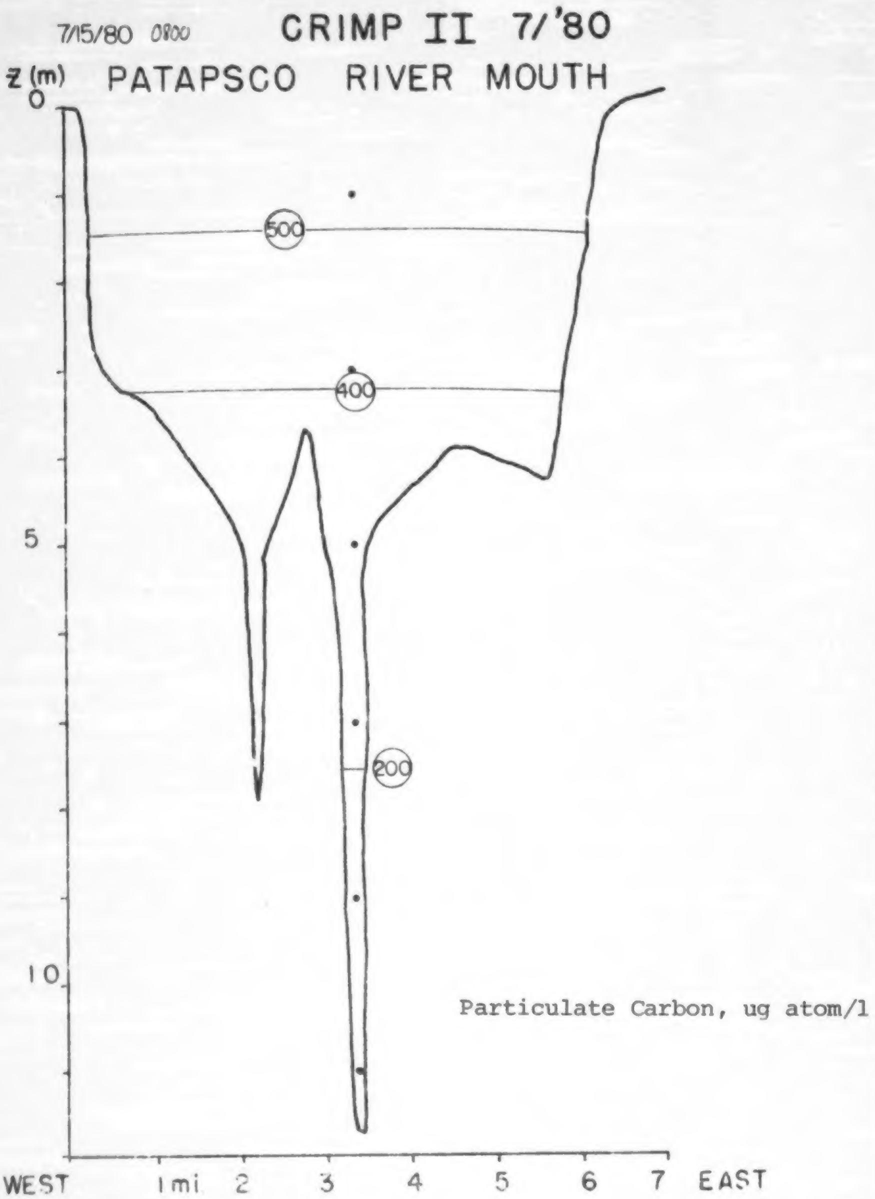

7/15/80 0645
CRIMP II 7/80
CHESAPEAKE BAY MOUTH
z
m)
5
0
5
24
25
26
27
28
29
30
24
28
29
Salinity, o/oo
WEST
M1
0.5
1
M2
2
M3
4
M4
6
7
M5
8
EAS
TRANSECT



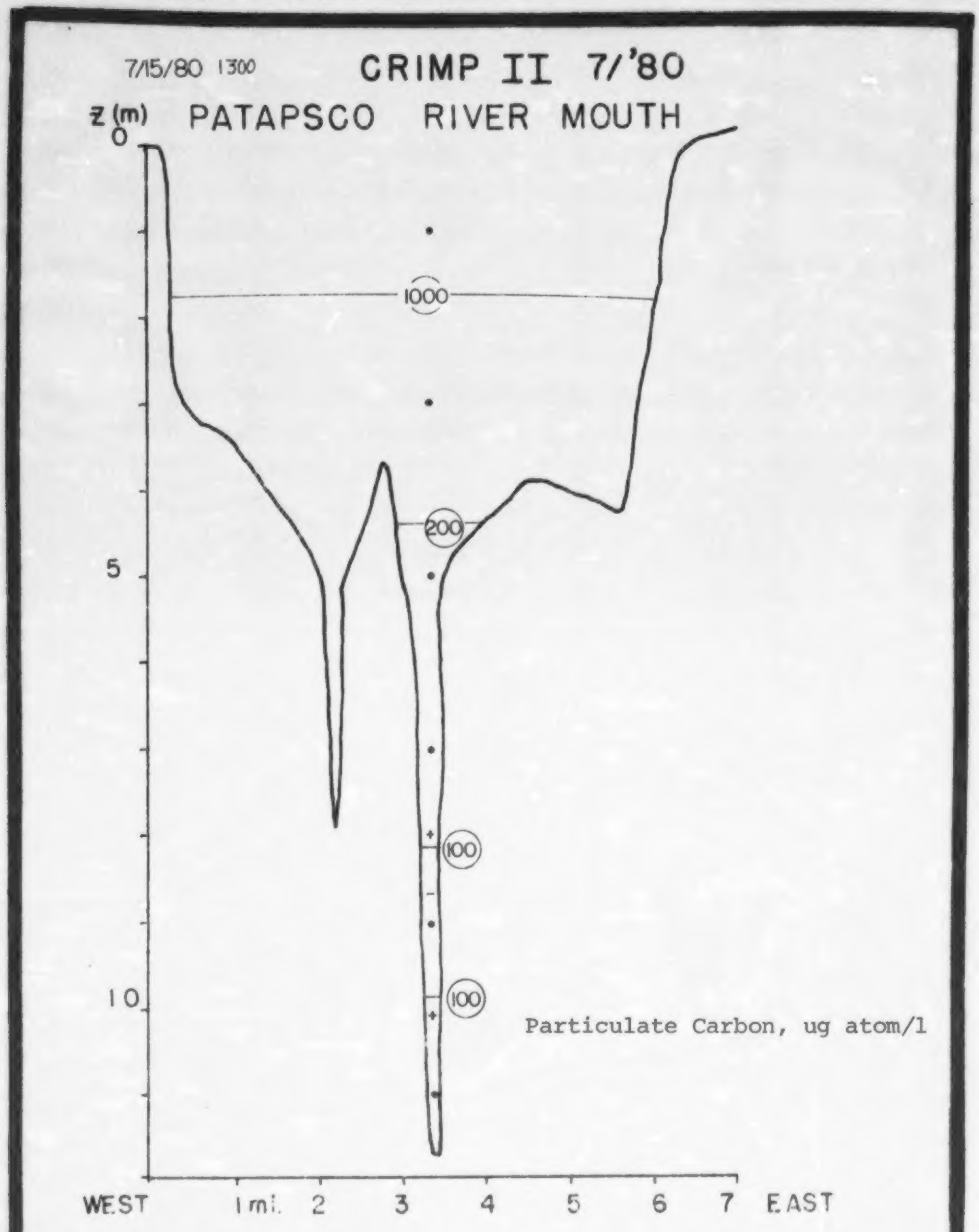
CRIMP II 7/80

CHESAPEAKE BAY MOUTH

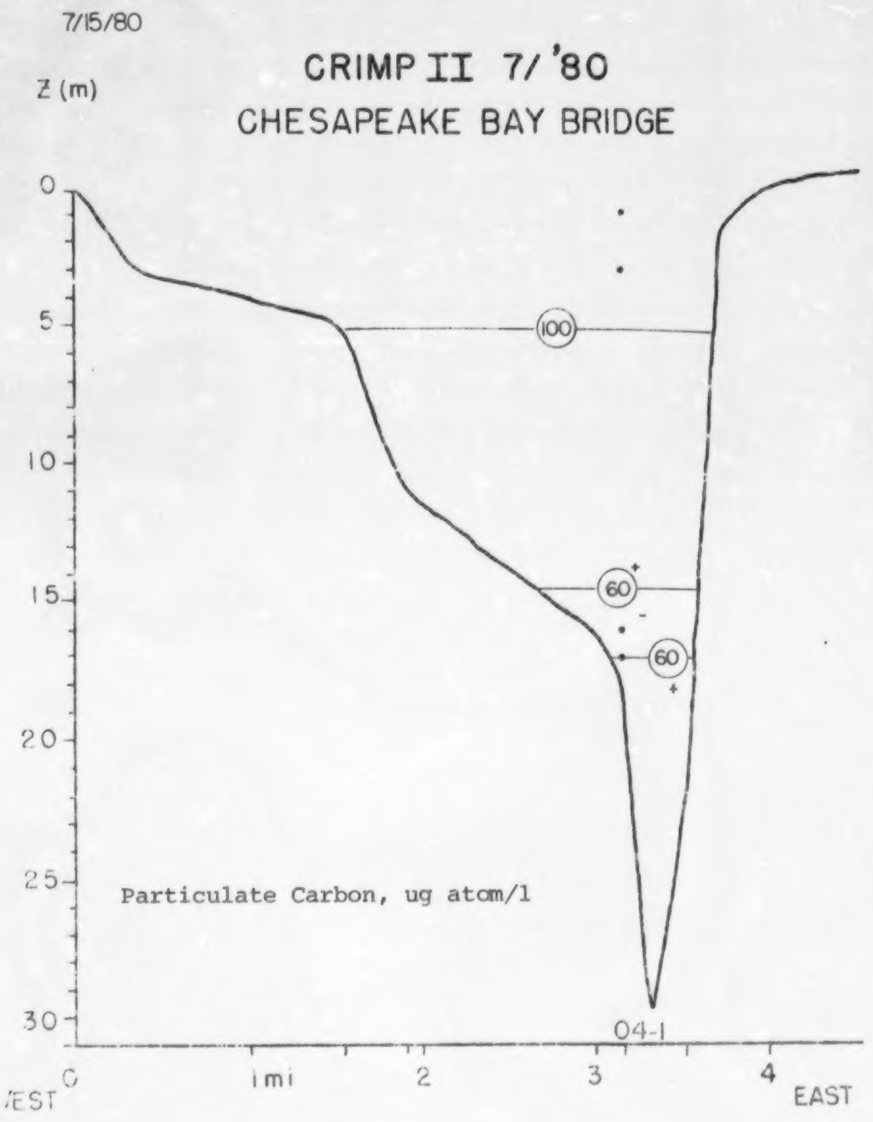
7/15/80 1105

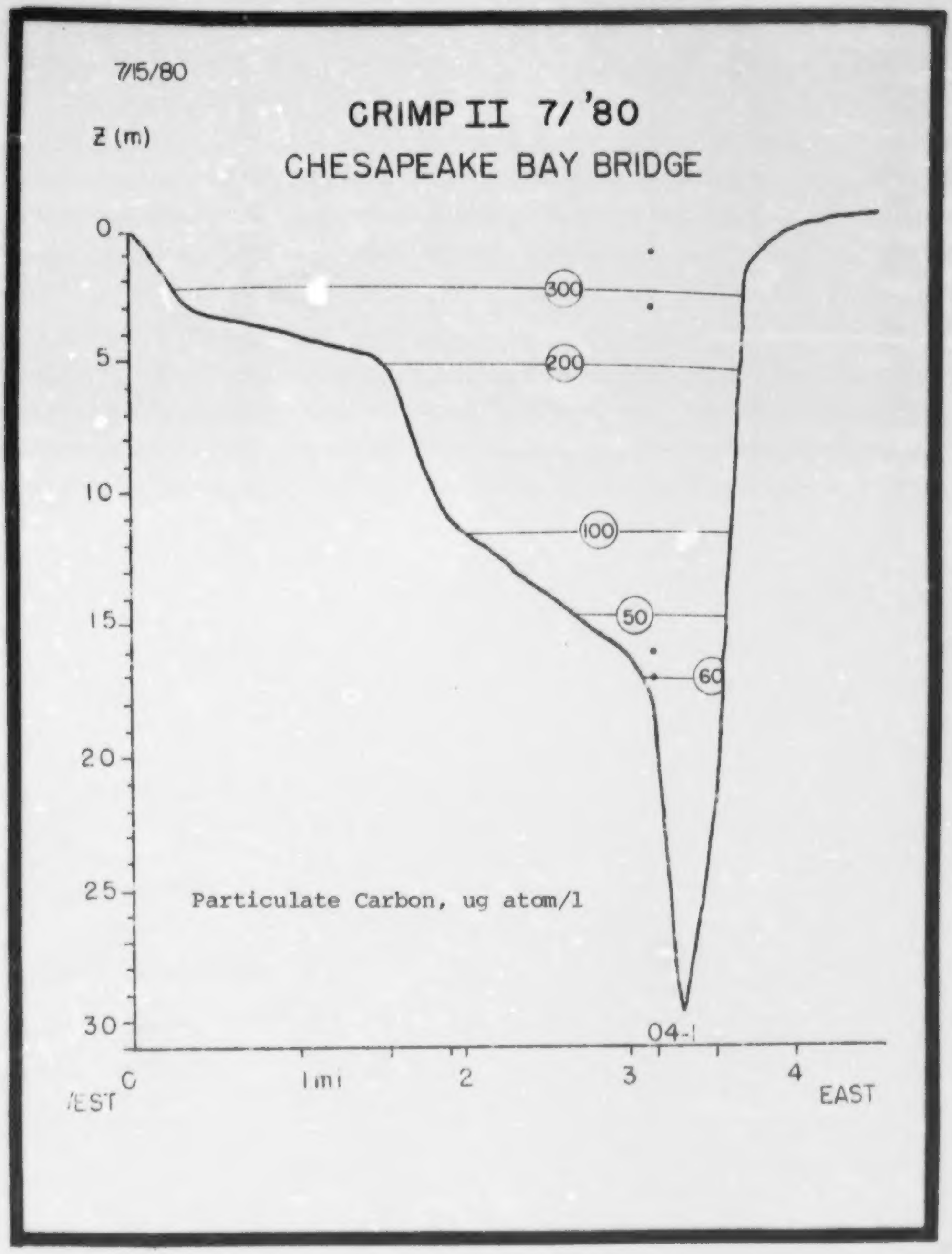


Particulate Carbon, ug atom/l



Particulate Carbon, ug atom/l


7/15/80
CRIMP II 7/'80
CHESAPEAKE BAY BRIDGE
Z (m)
0
5
10
15
20
25
30
100
60
60
+
-
+
Particulate Carbon, ug atom/l
04-1
0
1 mi
2
3
4
/EST
EAST

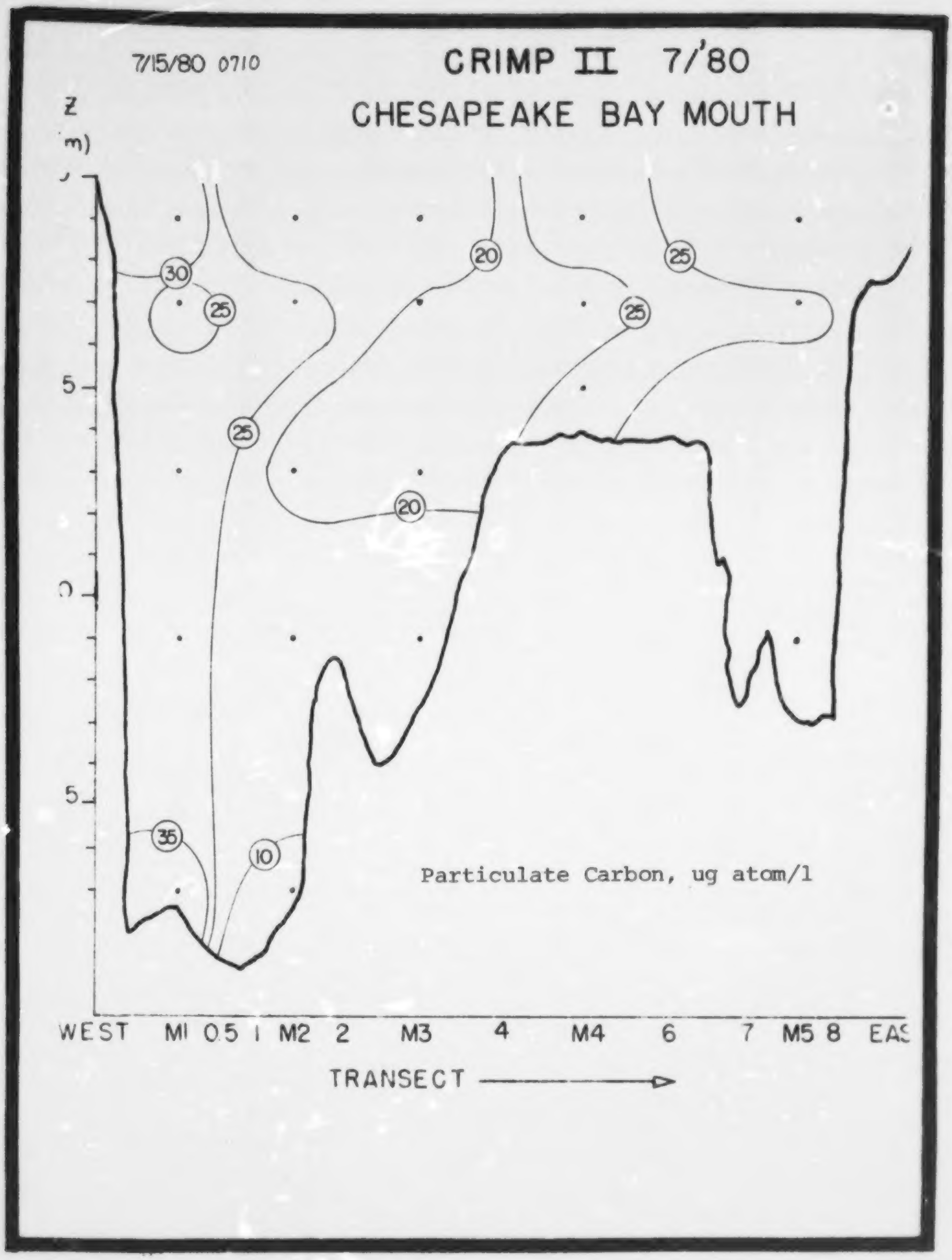


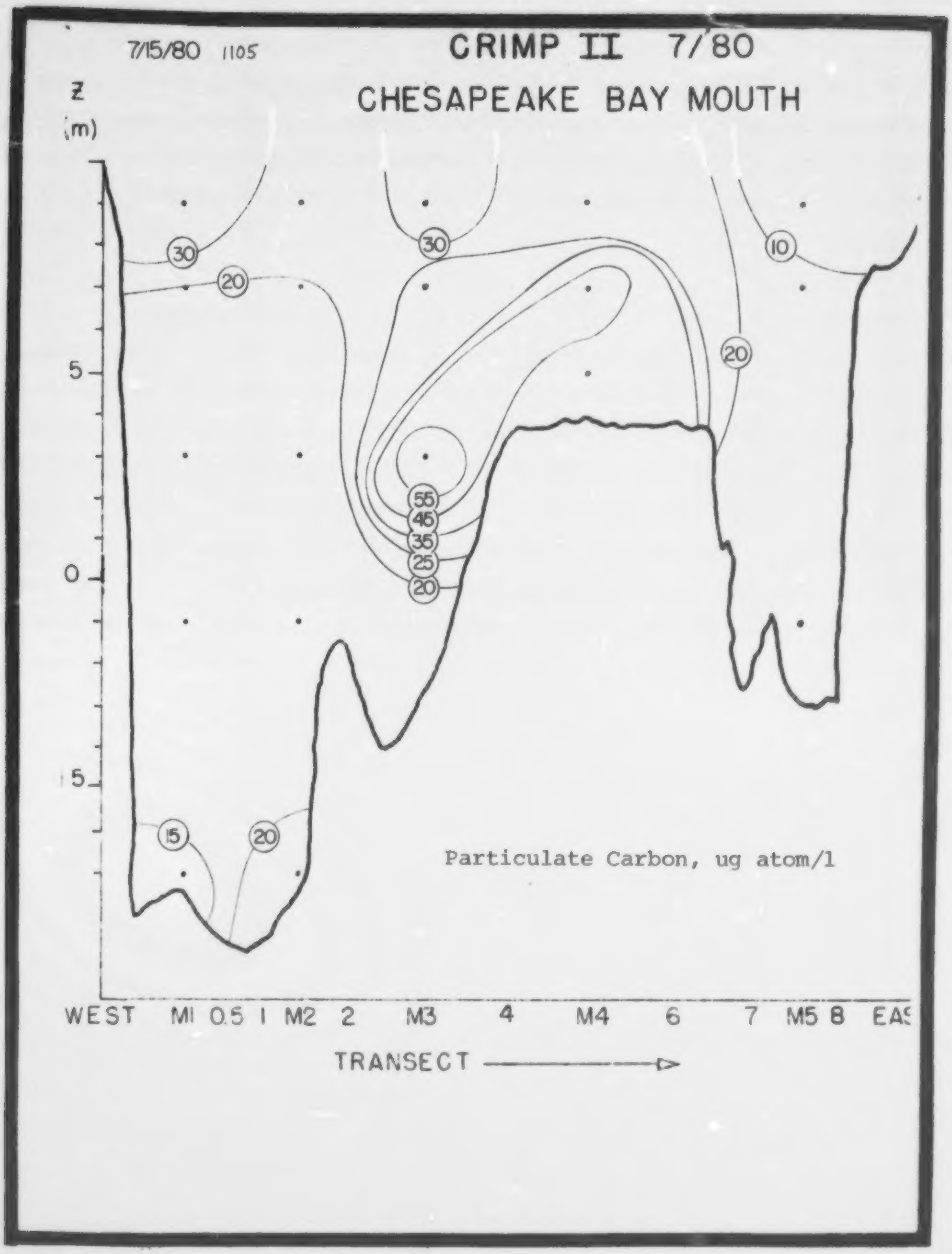
7/15/80

CRIMP II 7/'80

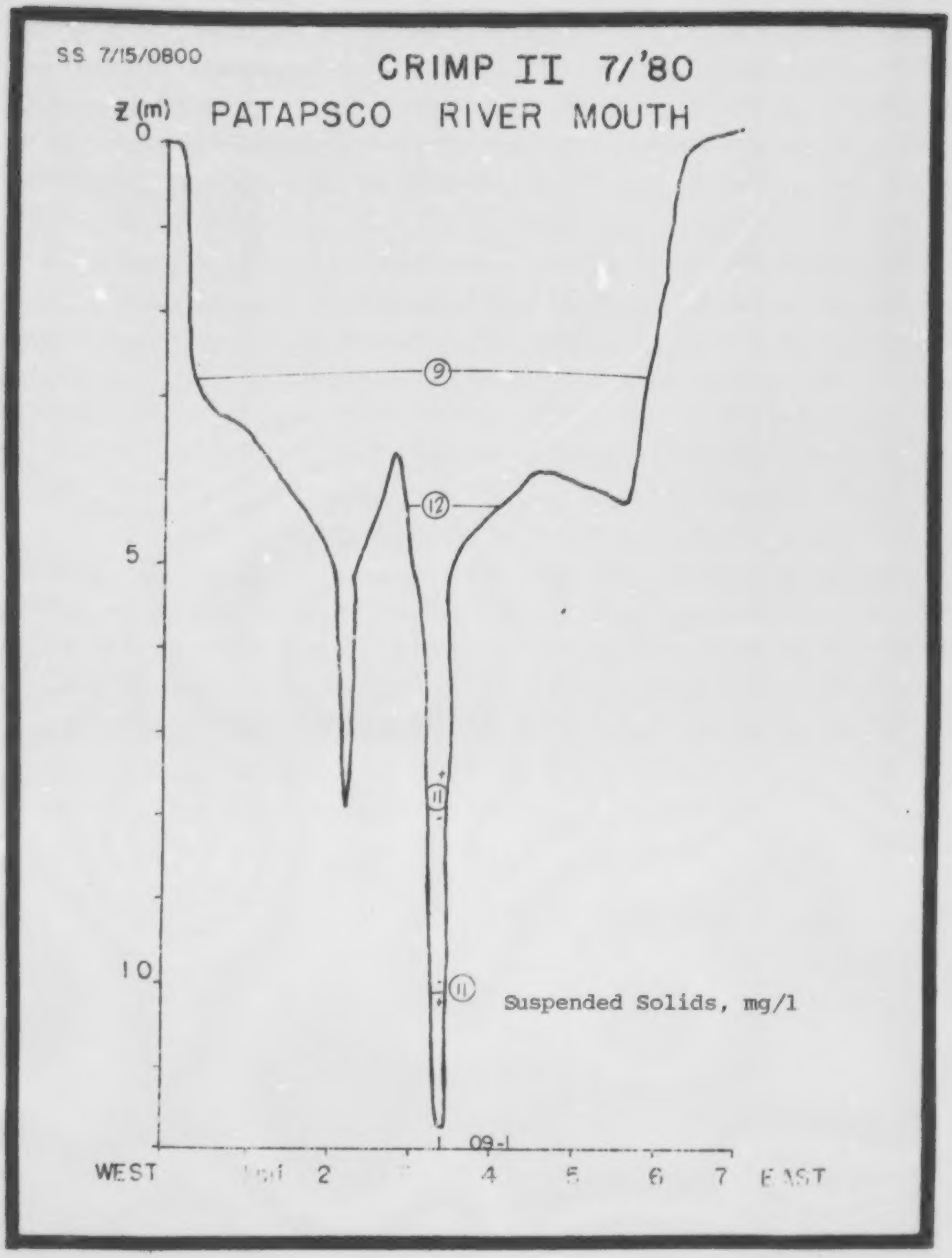
CHESAPEAKE BAY BRIDGE

Particulate Carbon, ug atom/l


7/15/80 0710
CRIMP II 7/'80
CHESAPEAKE BAY MOUTH
Z
m)
5
0
5
30
25
25
20
25
25
20
25
35
10
Particulate Carbon, ug atom/l
WEST
MI
0.5
1
M2
2
M3
4
M4
6
7
M5
8
EAS
TRANSECT



Particulate Carbon, ug atom/l



Suspended Solids, mg/l

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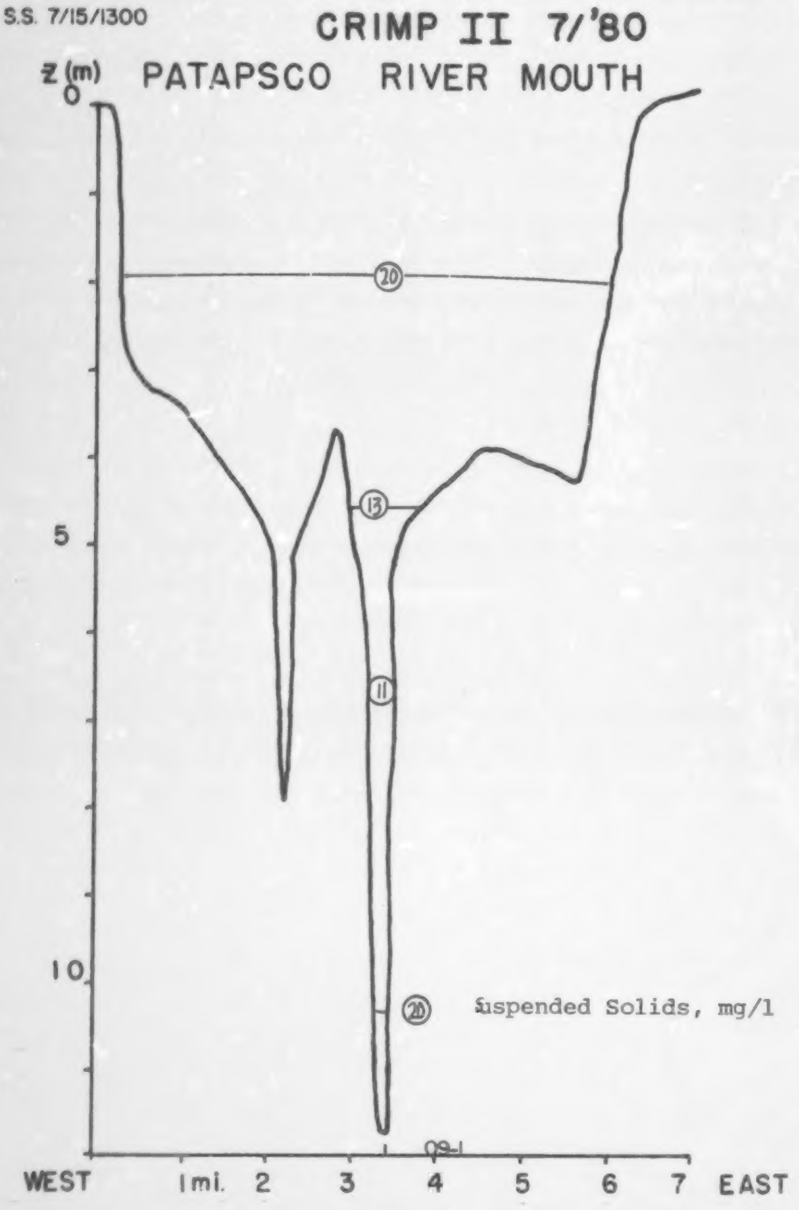
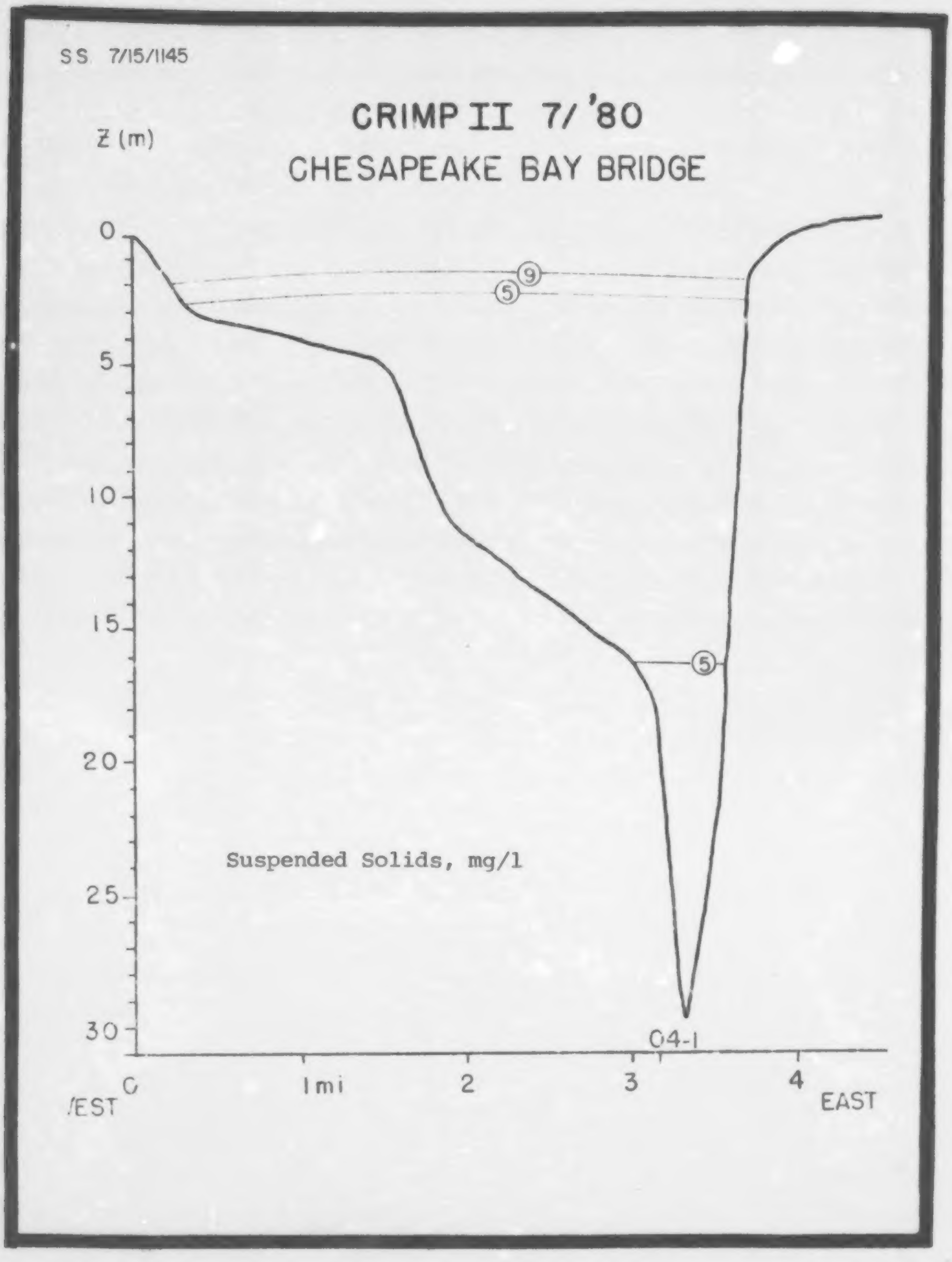


CRIMP II 7/'80

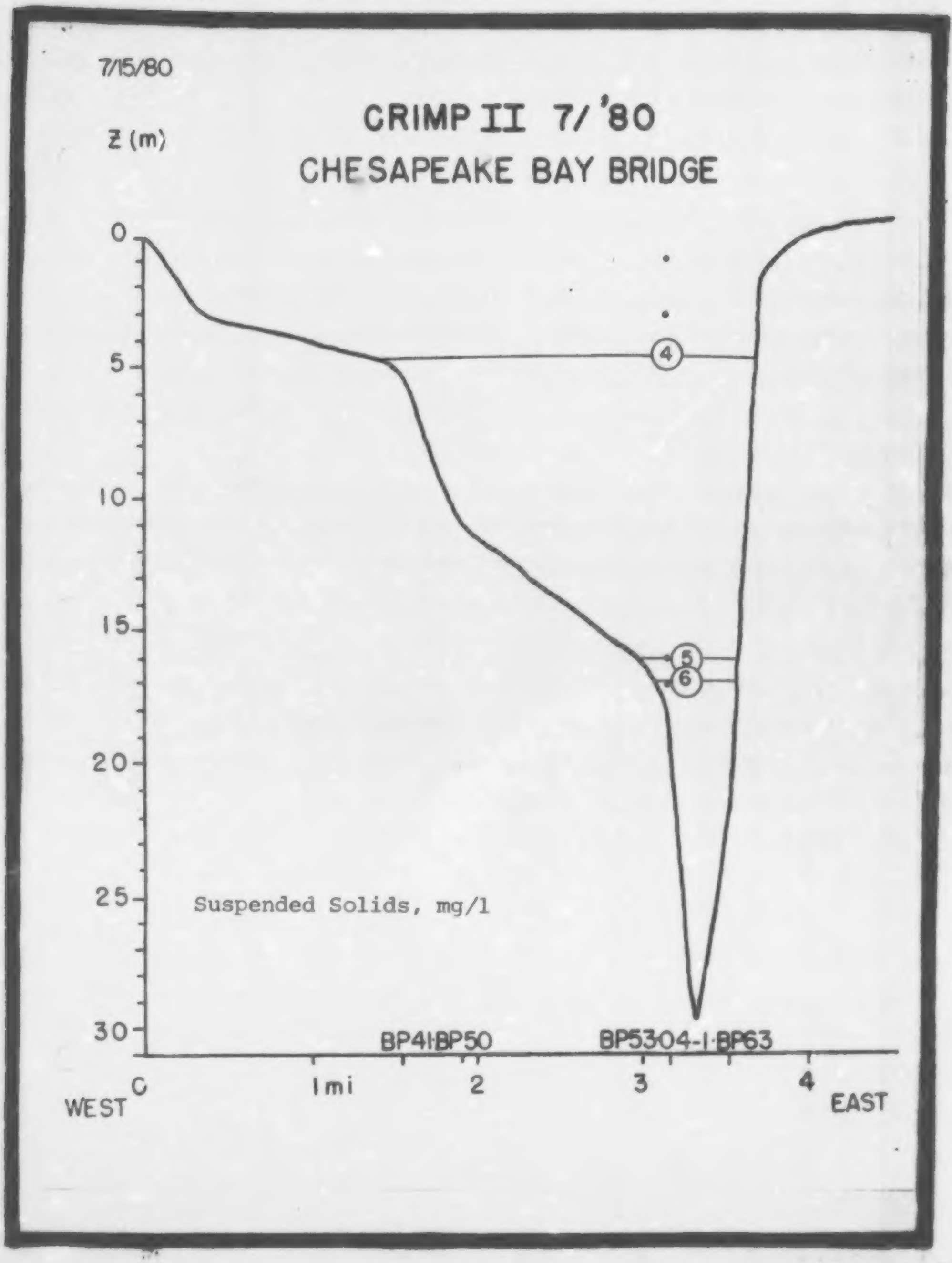
PATAPSCO RIVER MOUTH

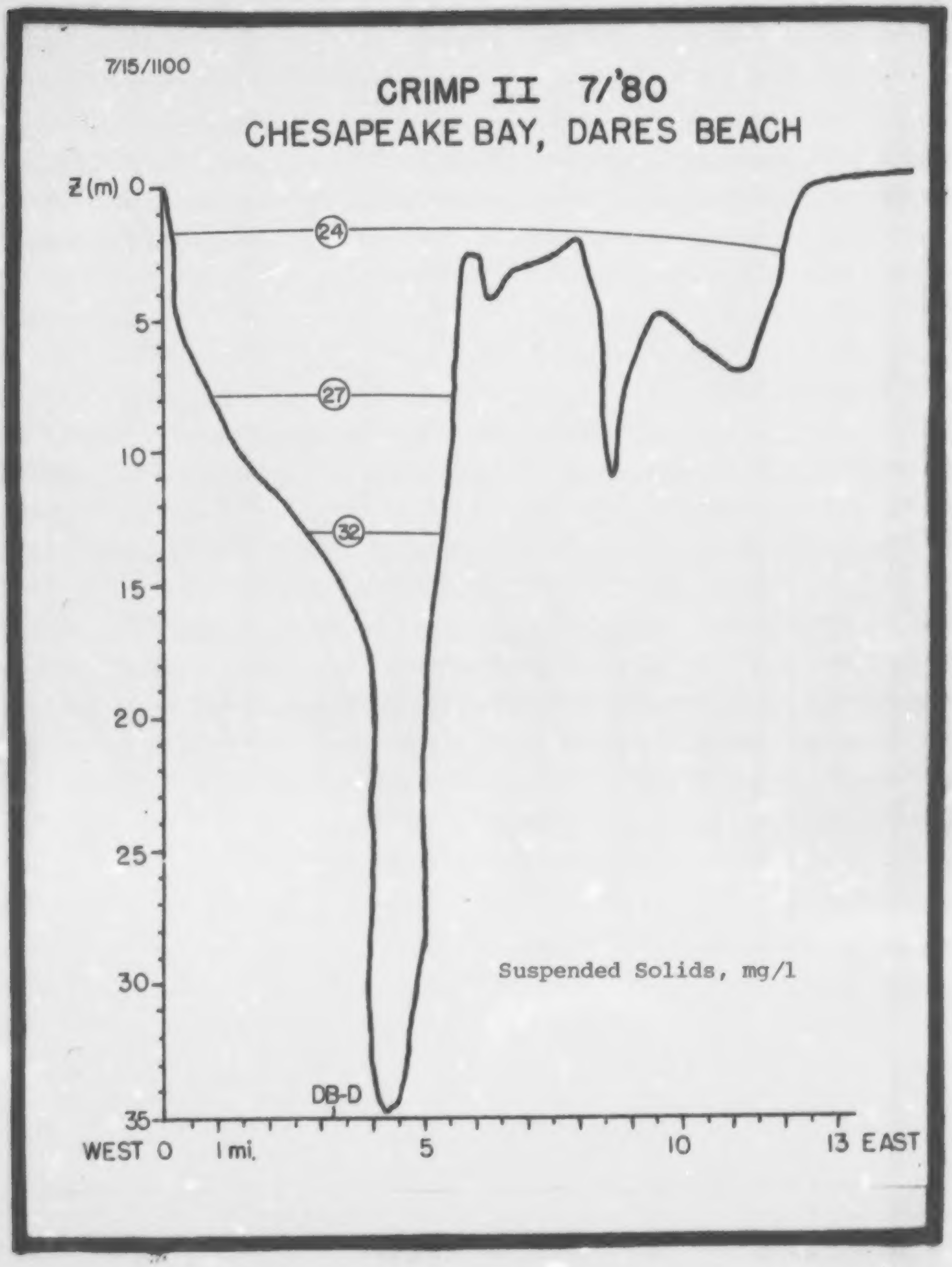

SS 7/15/1145

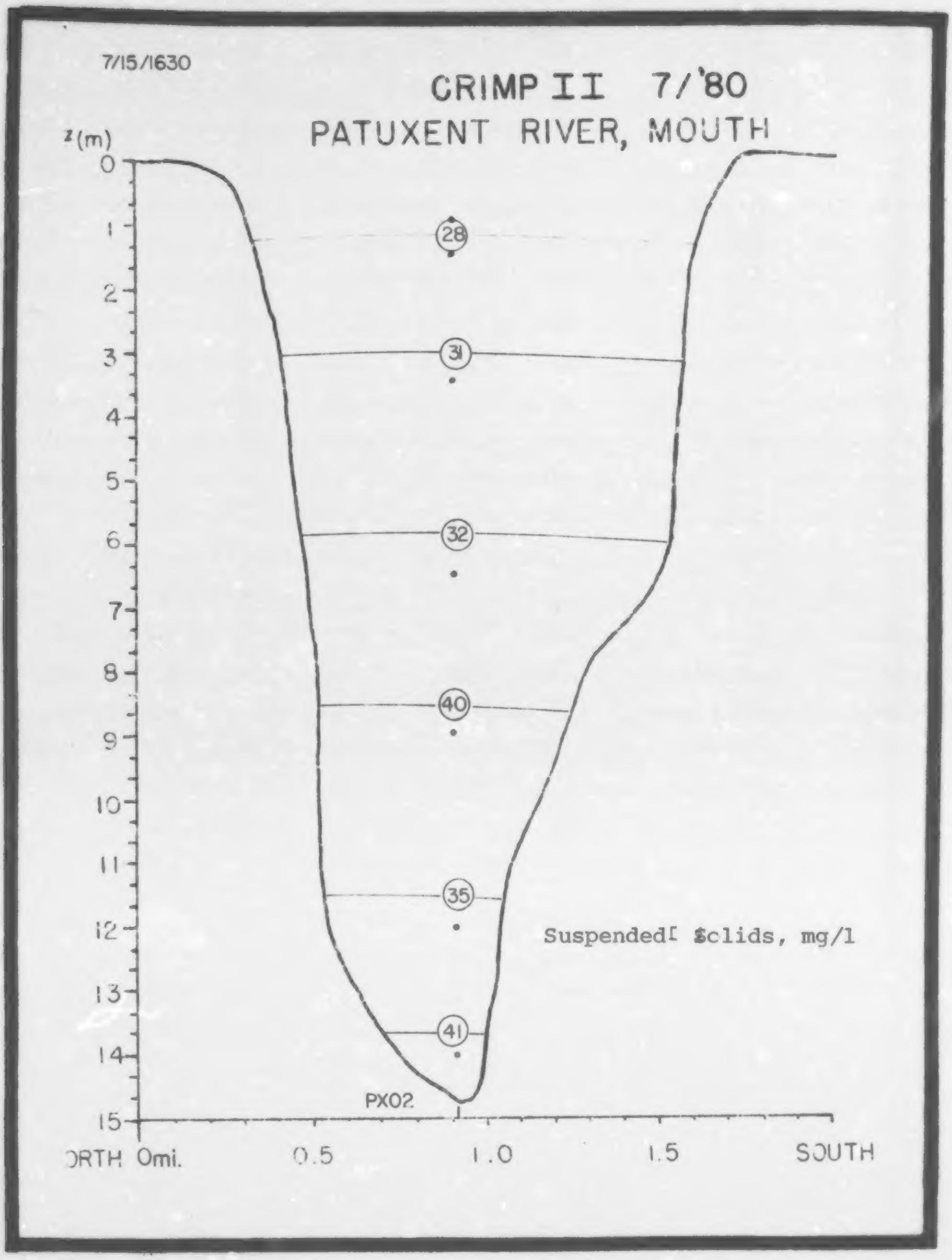
CRIMP II 7/'80

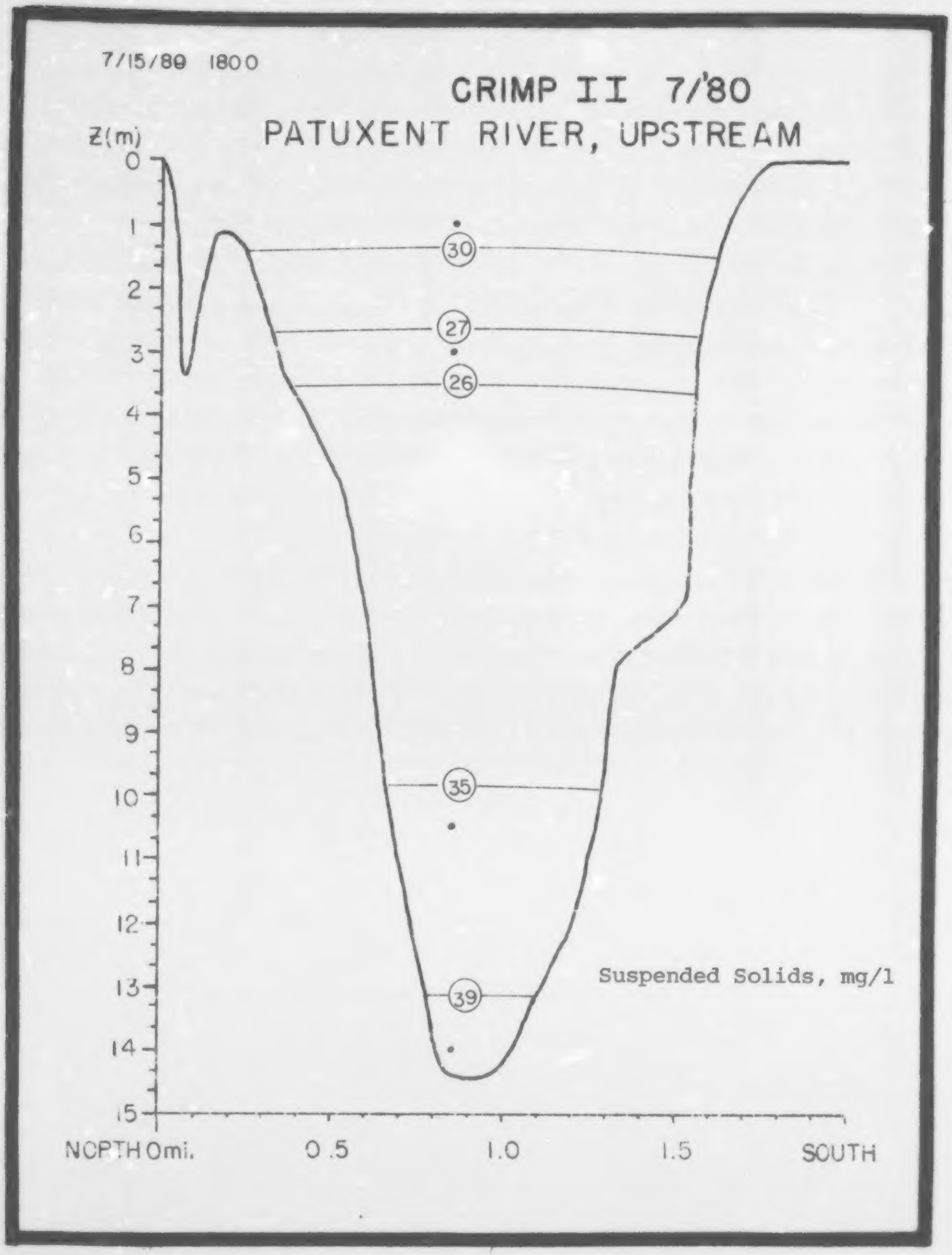
CHESAPEAKE BAY BRIDGE

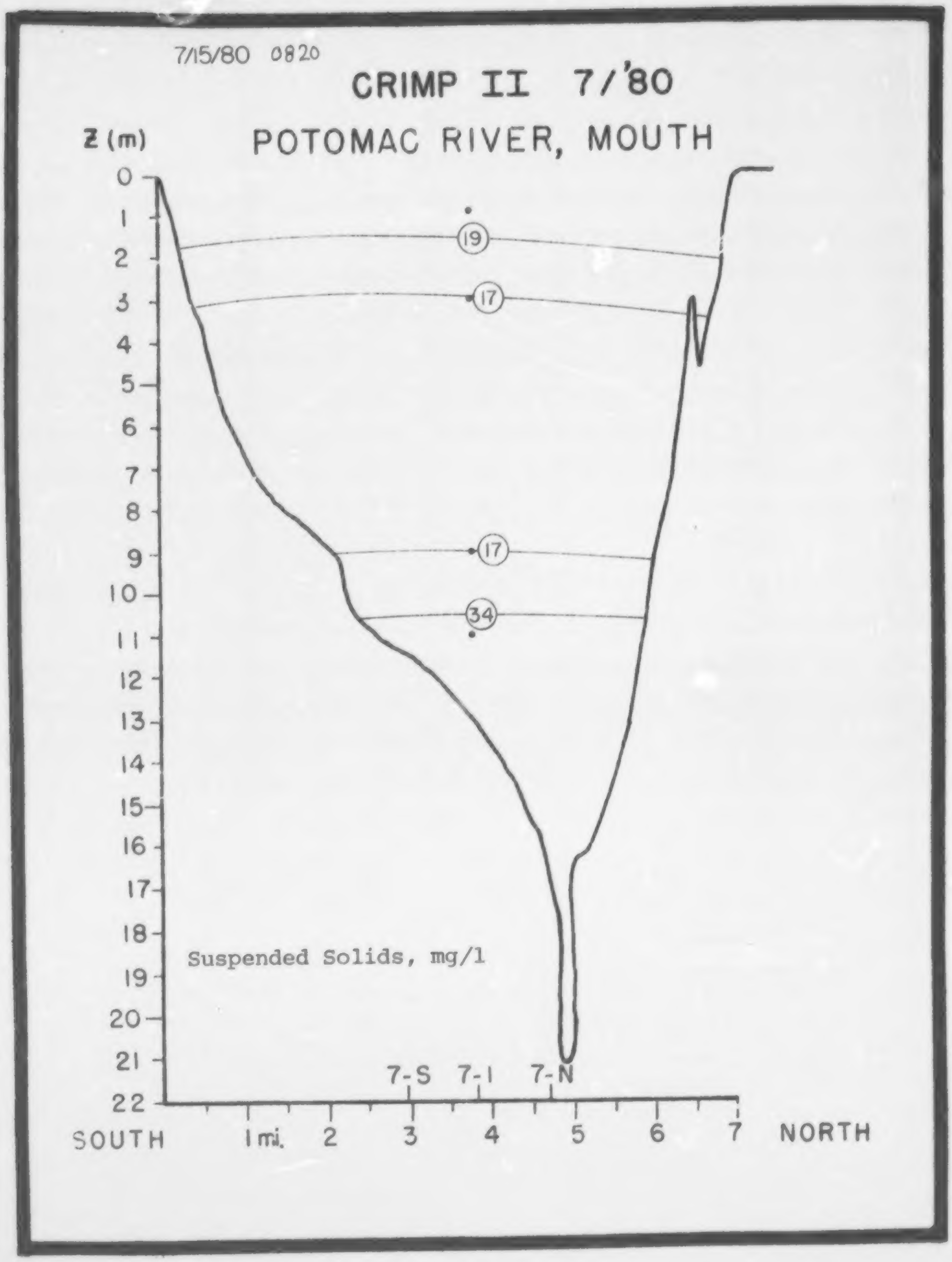
Suspended Solids, mg/l

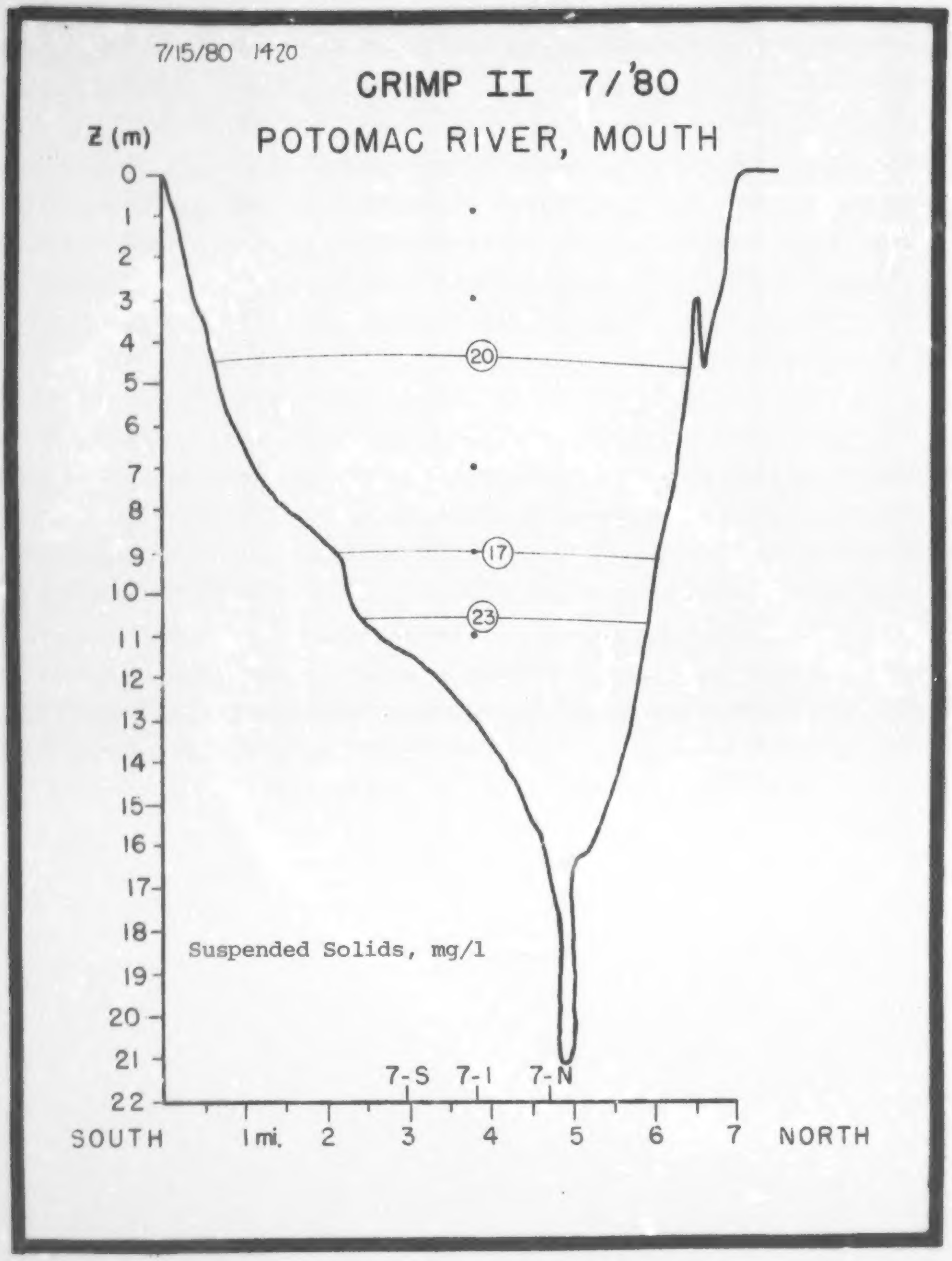

7/15/80
CRIMP II 7/'80
CHESAPEAKE BAY BRIDGE
Z (m)
0
5
10
15
20
25
30
4
5
6
Suspended Solids, mg/l
BP41·BP50
BP5304-1·BP63
0
1mi
2
3
4
WEST
EAST

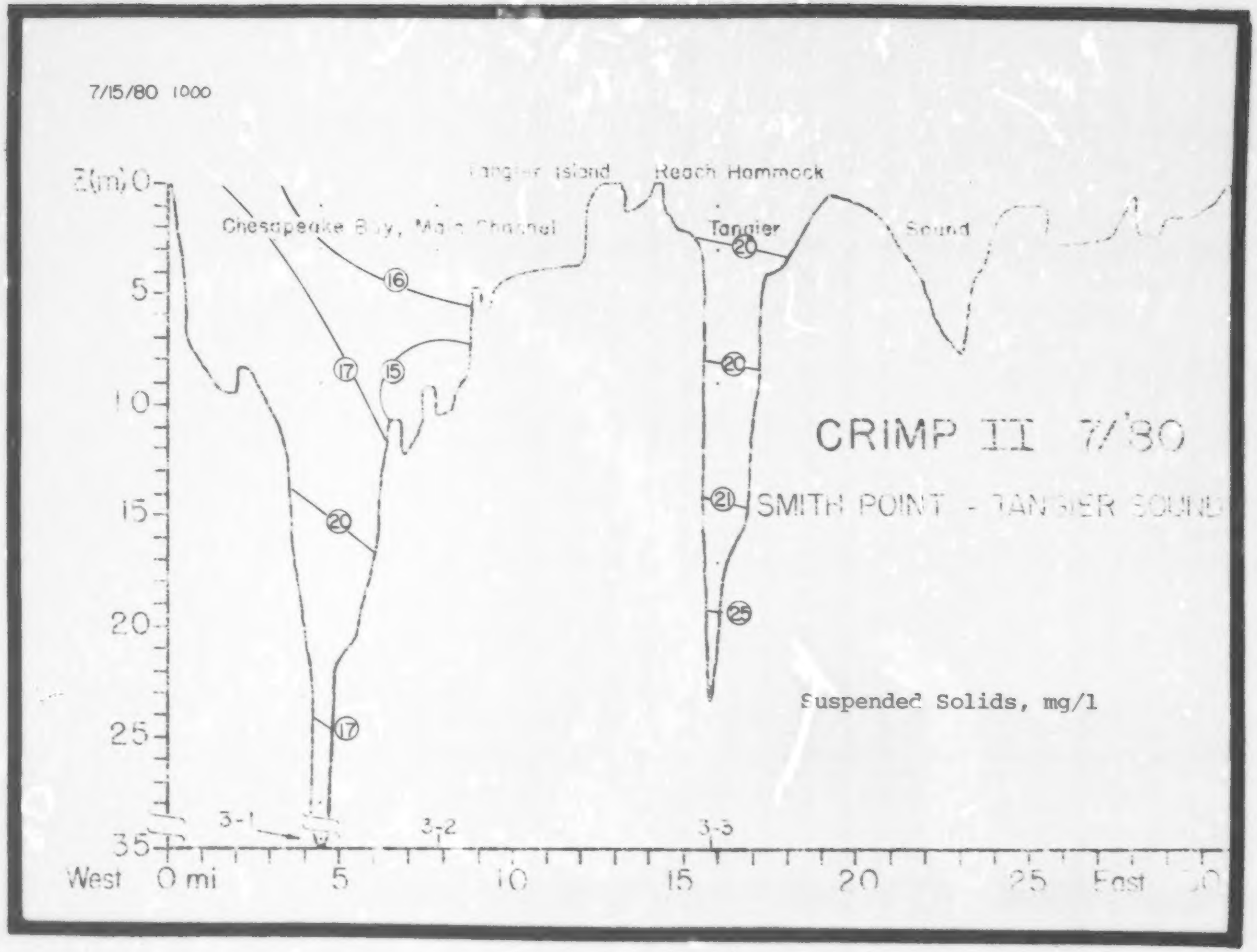

7/15/1100
CRIMP II 7/'80
CHESAPEAKE BAY, DARES BEACH
Z (m) 0
5
10
15
20
25
30
35
24
27
32
DB-D
WEST 0
1 mi.
5
10
13 EAST
Suspended Solids, mg/l


7/15/1630
CRIMP II 7/'80
PATUXENT RIVER, MOUTH
z(m)
0
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
28
31
32
40
35
41
PX02
Suspended Solids, mg/l
ORTH 0mi.
0.5
1.0
1.5
SOUTH

7/15/80 1800
CRIMP II 7/'80
PATUXENT RIVER, UPSTREAM
Z(m)
0
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
30
27
26
35
39
Suspended Solids, mg/l
NORTH 0mi.
0.5
1.0
1.5
SOUTH


7/15/80 0820
CRIMP II 7/'80
POTOMAC RIVER, MOUTH
Z (m)
0
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
19
17
17
34
Suspended Solids, mg/l
7-S
7-1
7-N
SOUTH
1 mi.
2
3
4
5
6
7
NORTH


7/15/80 1420
CRIMP II 7/'80
POTOMAC RIVER, MOUTH
Z (m)
0
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
20
17
23
Suspended Solids, mg/l
7-S
7-I
7-N
SOUTH
1 mi.
2
3
4
5
6
7
NORTH



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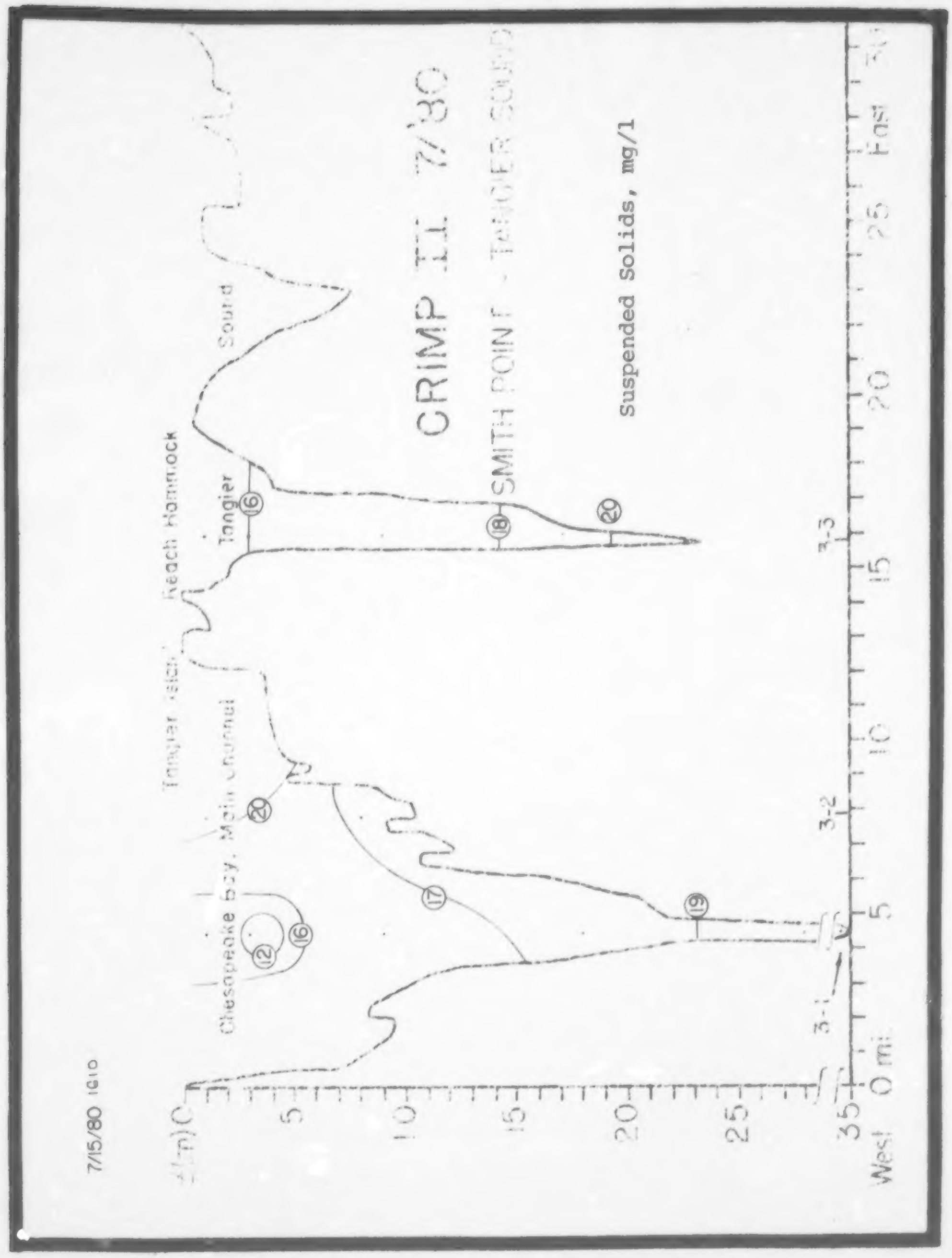
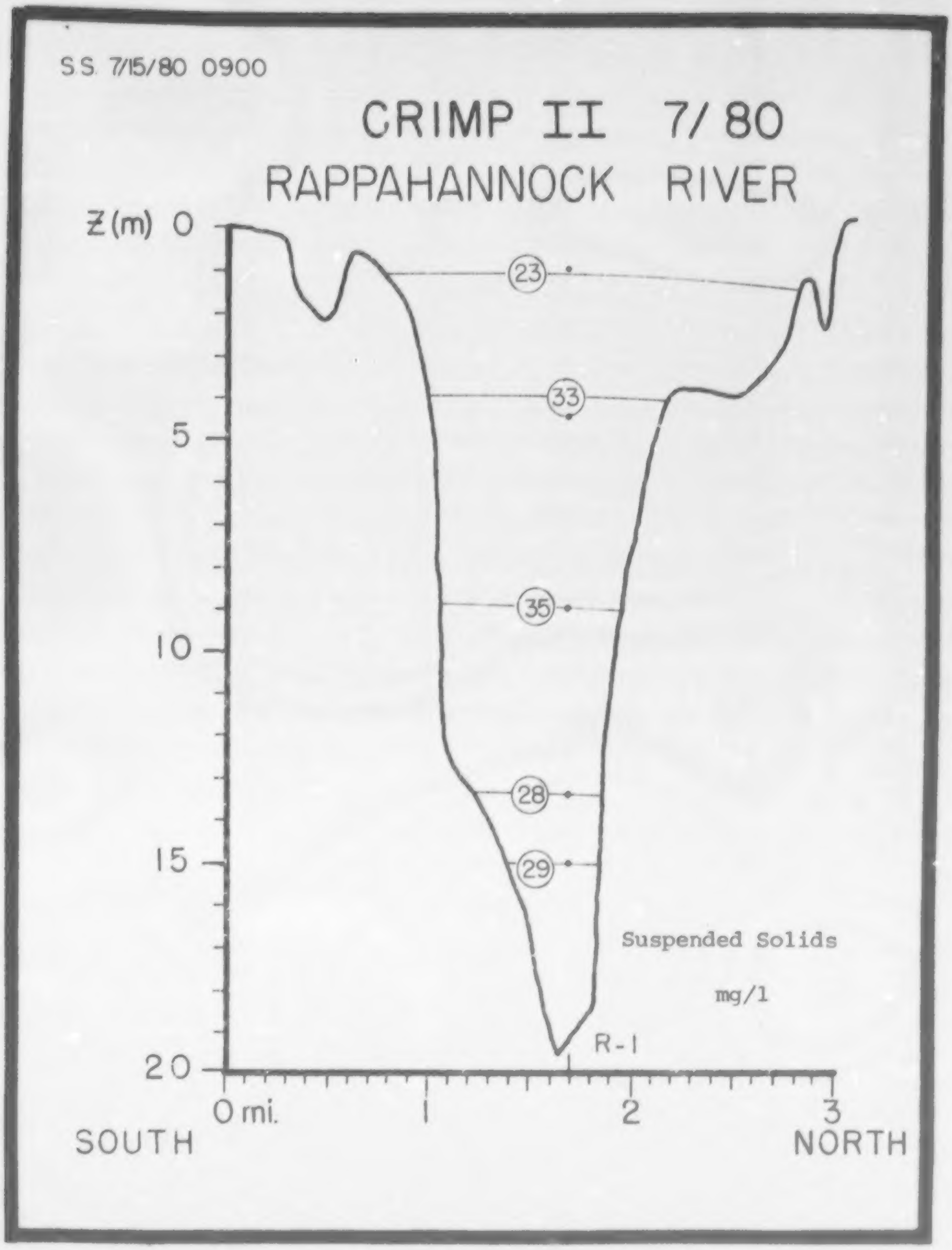
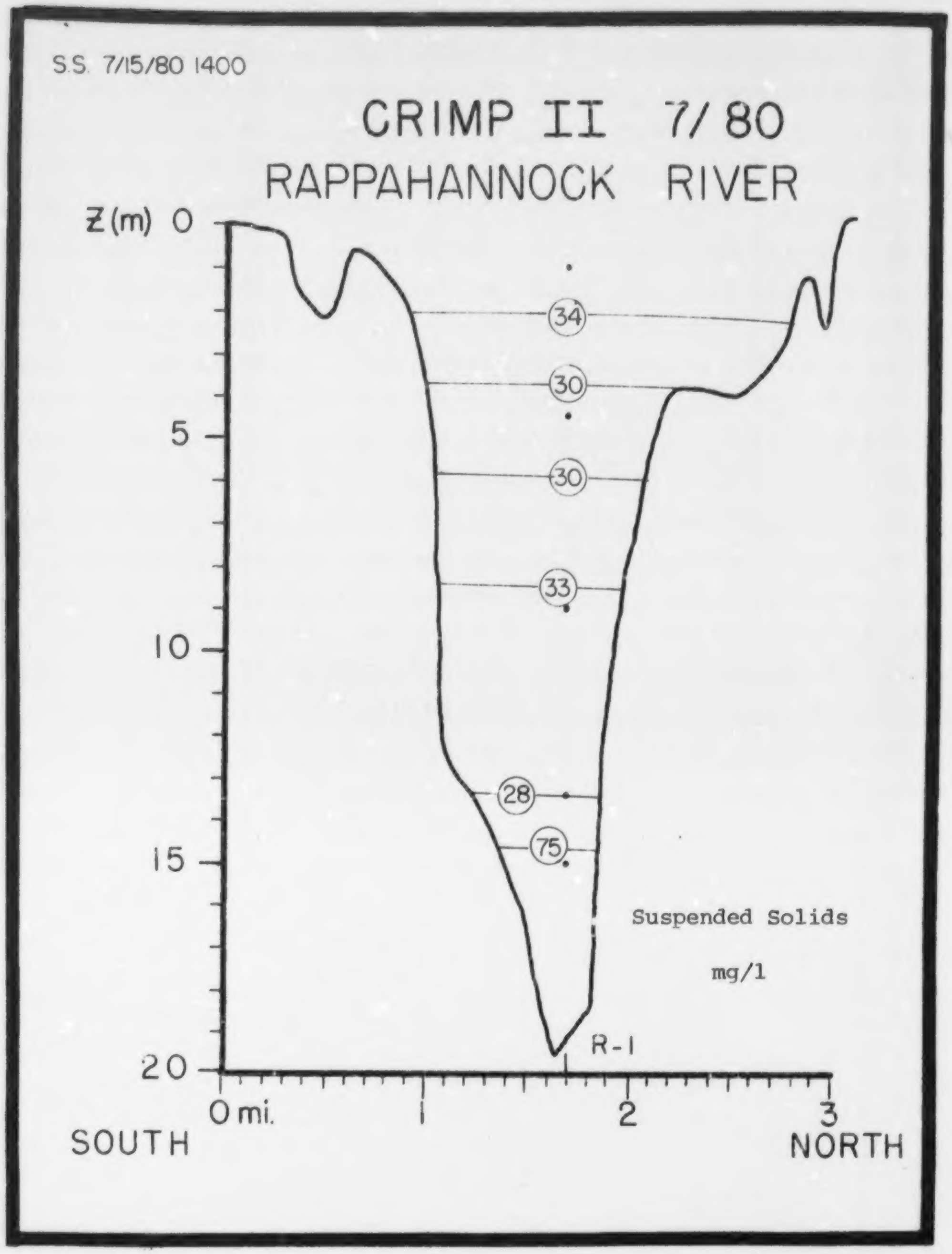


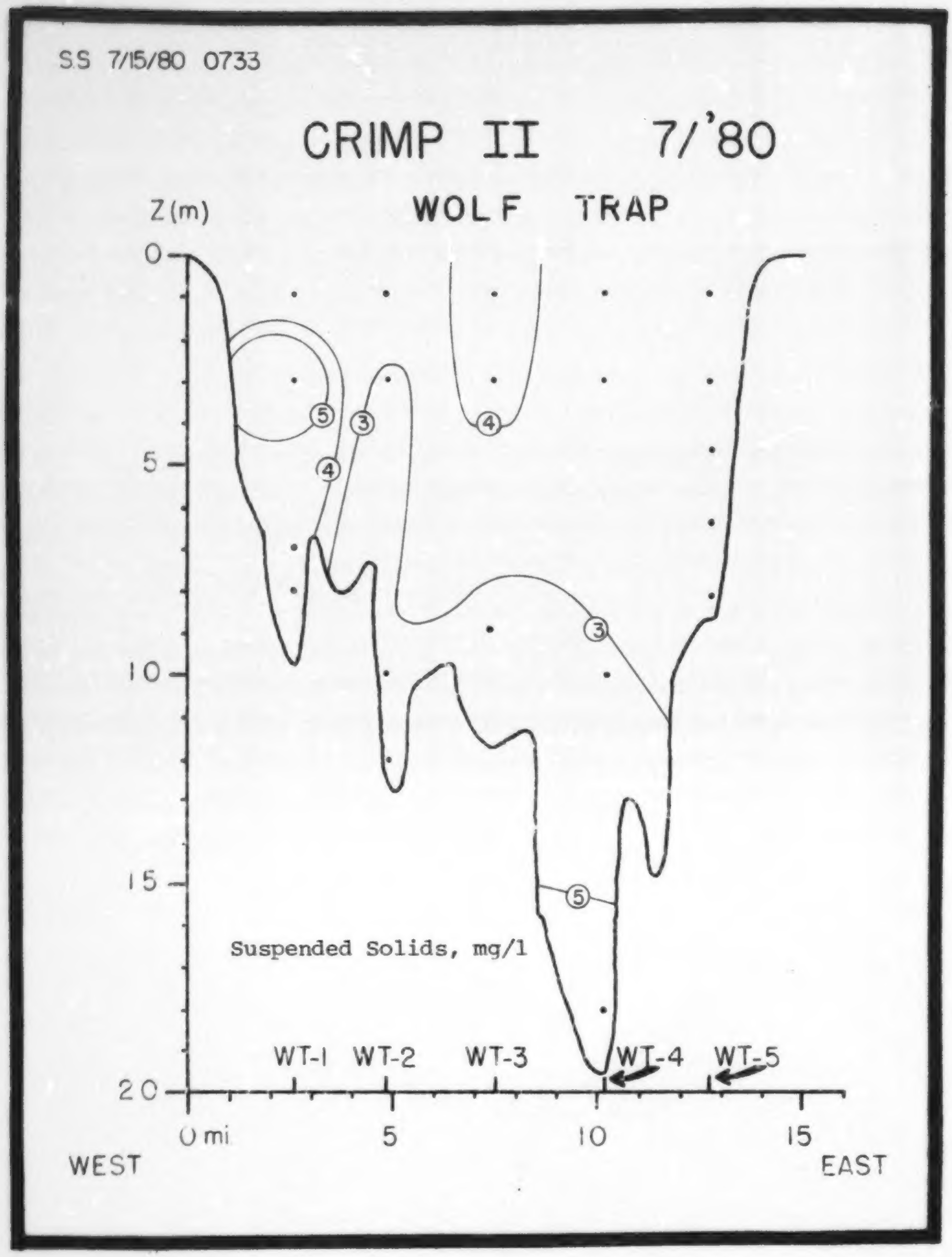
CRIMP II 7/'80

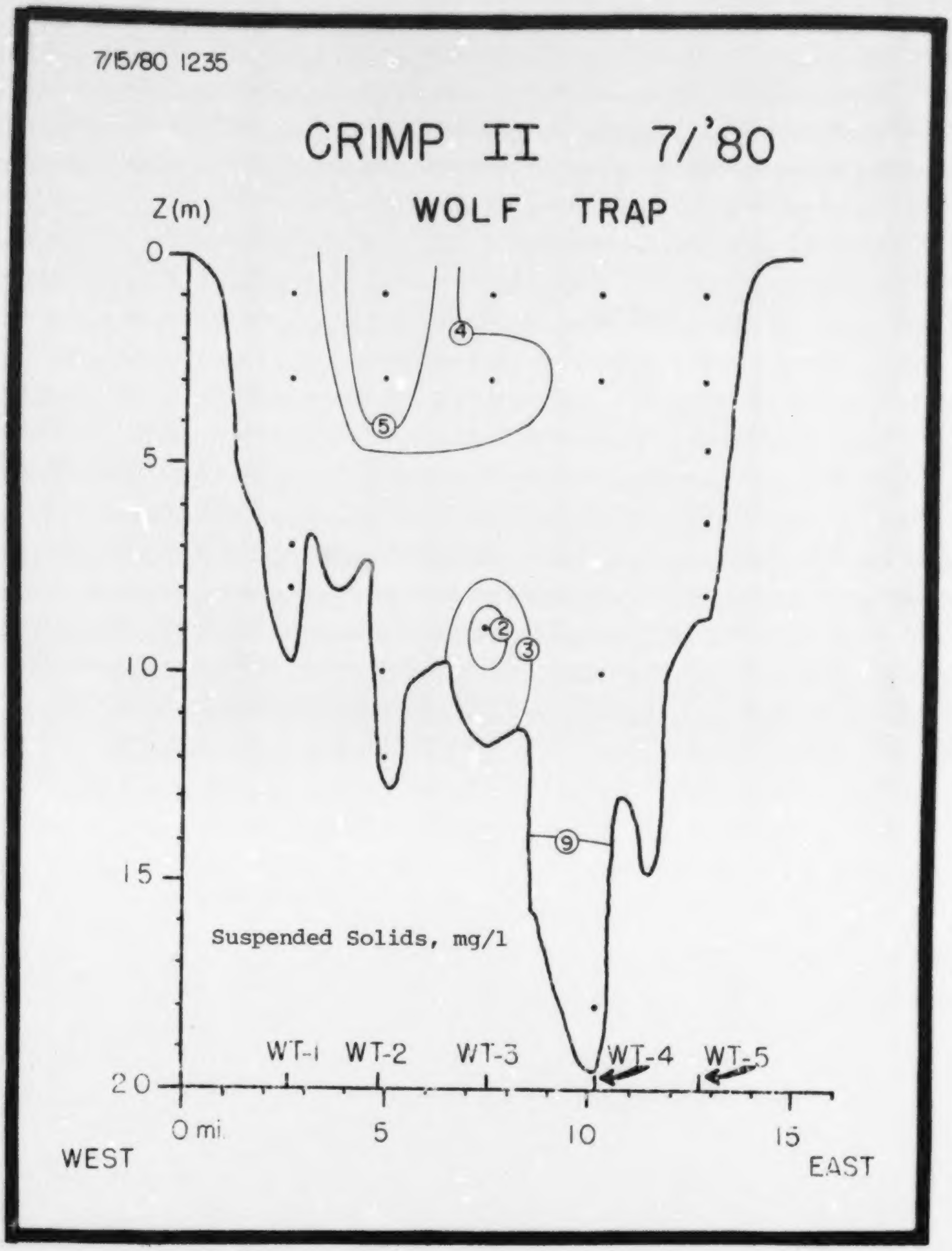
SMITH POINT - TANGIER SOUND

Suspended Solids, mg/1


S.S. 7/15/80 0900
CRIMP II 7/80
RAPPAHANNOCK RIVER
Z(m) 0
5
10
15
20
23
33
35
28
29
Suspended Solids
mg/1
R-1
0 mi.
1
2
3
SOUTH
NORTH


S.S. 7/15/80 1400
CRIMP II 7/80
RAPPAHANNOCK RIVER
Z(m) 0
5
10
15
20
34
30
30
33
28
75
R-1
0 mi.
1
2
3
SOUTH
NORTH
Suspended Solids
mg/1


SS 7/15/80 0733
CRIMP II 7/'80
WOLF TRAP
Z(m)
0
5
10
15
20
5
3
4
3
4
3
5
Suspended Solids, mg/l
WT-1
WT-2
WT-3
WT-4
WT-5
0 mi
5
10
15
WEST
EAST

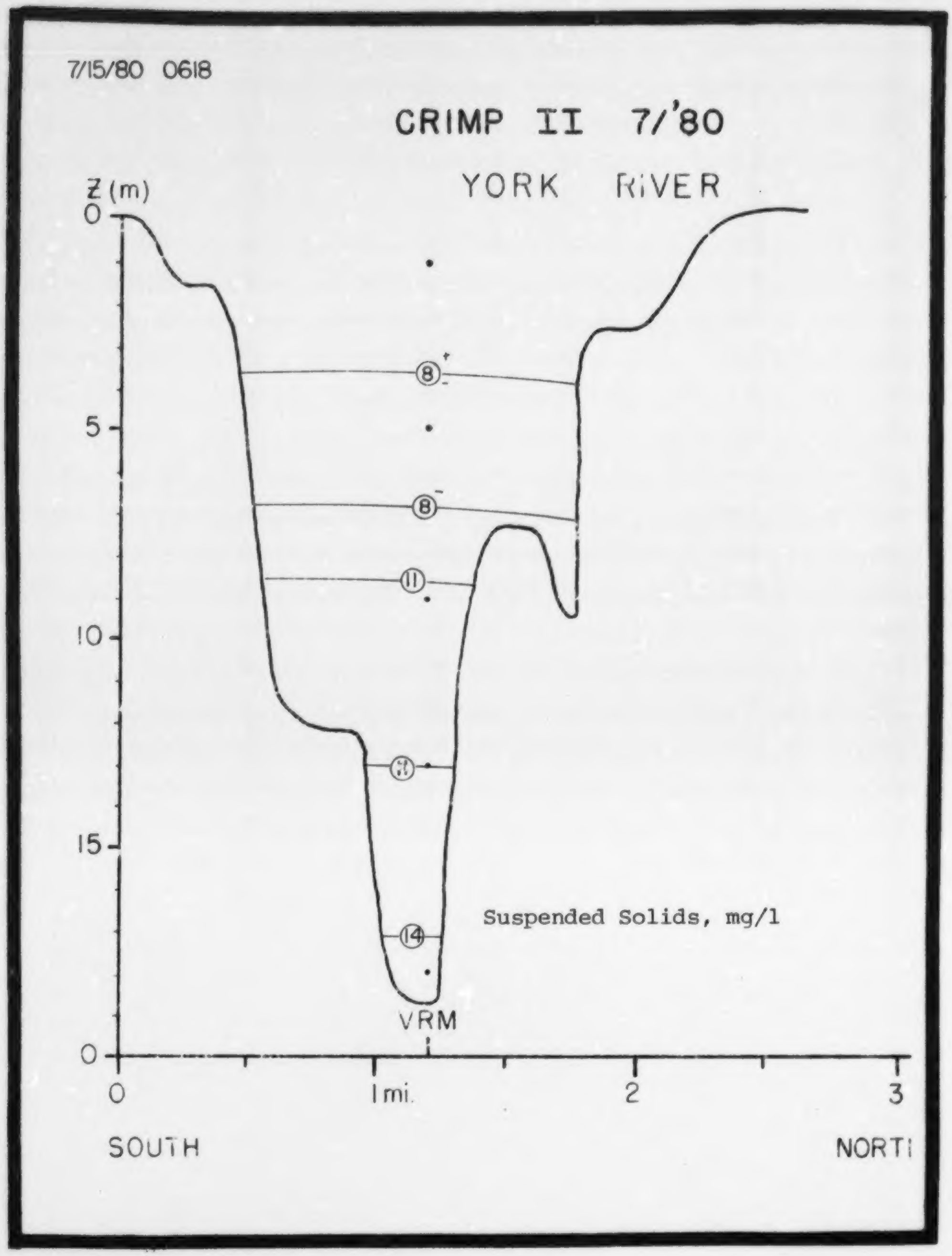


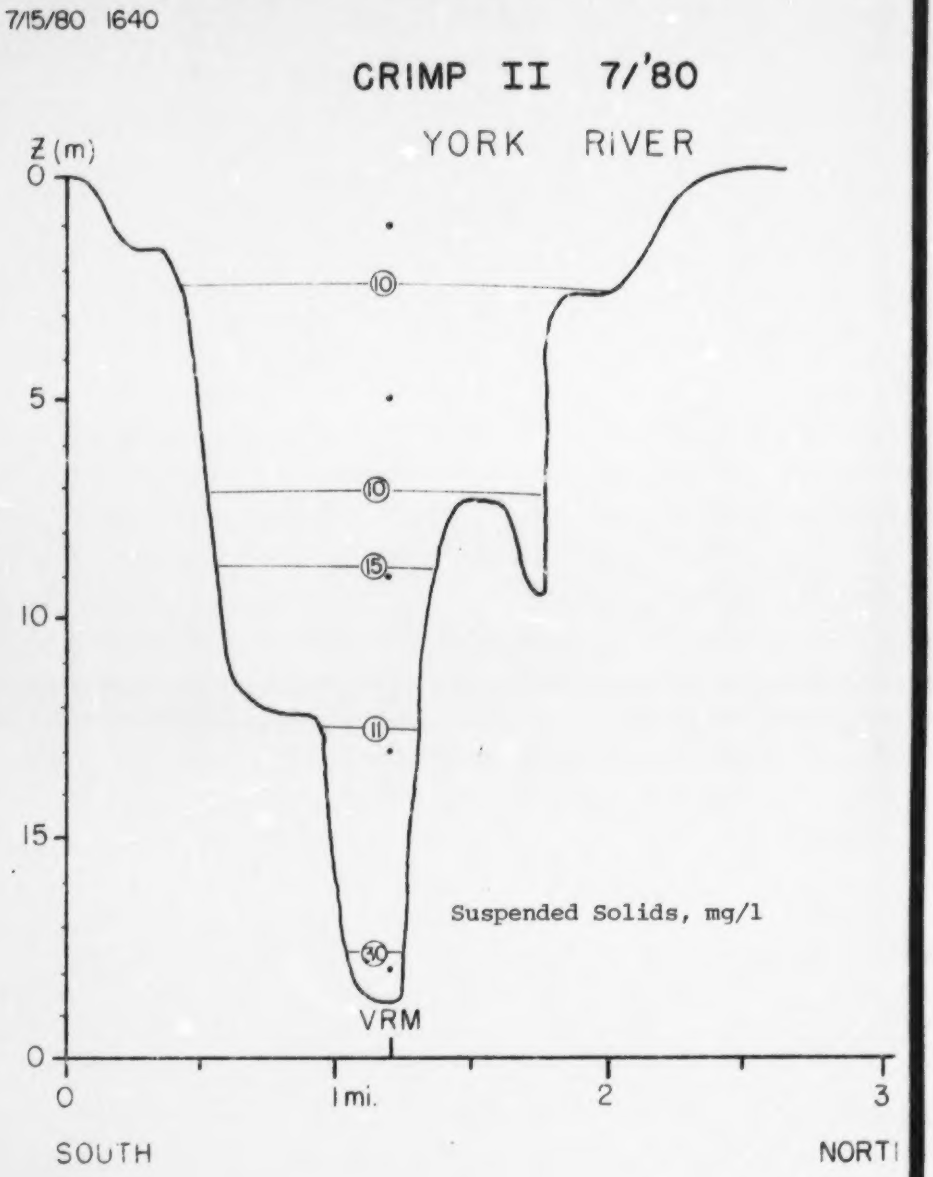
7/15/80 1235

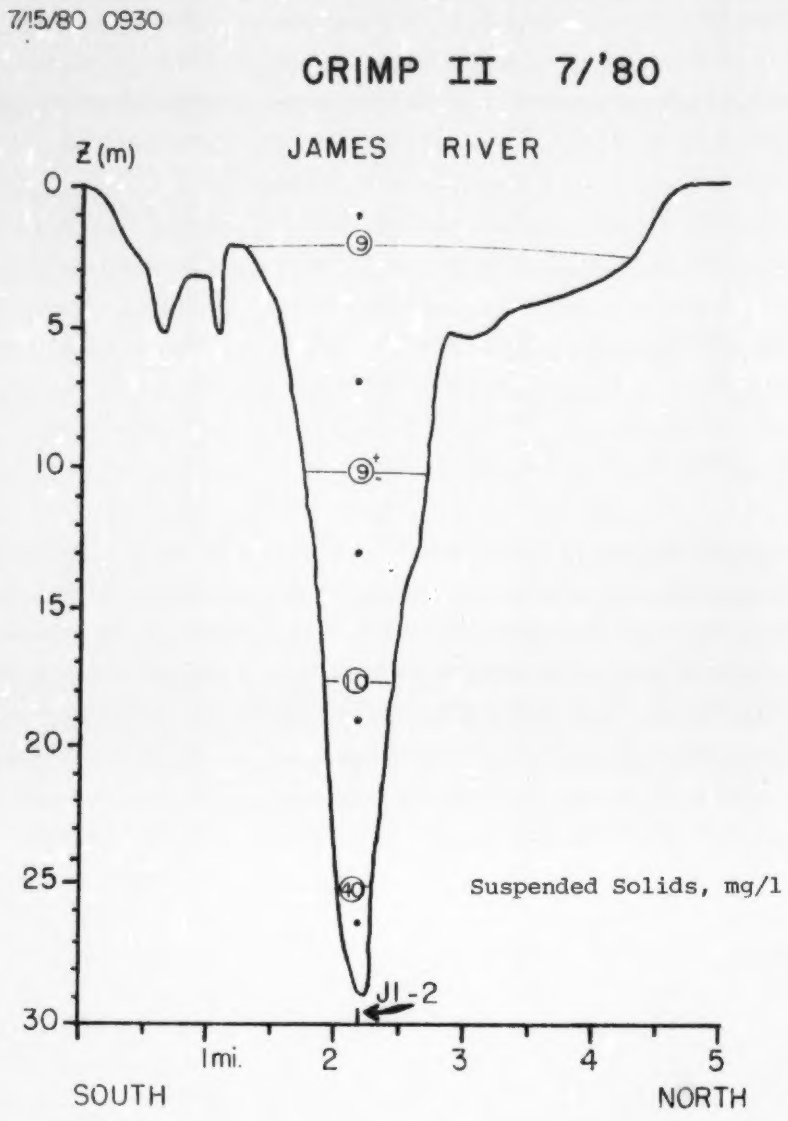
CRIMP II 7/'80

WOLF TRAP

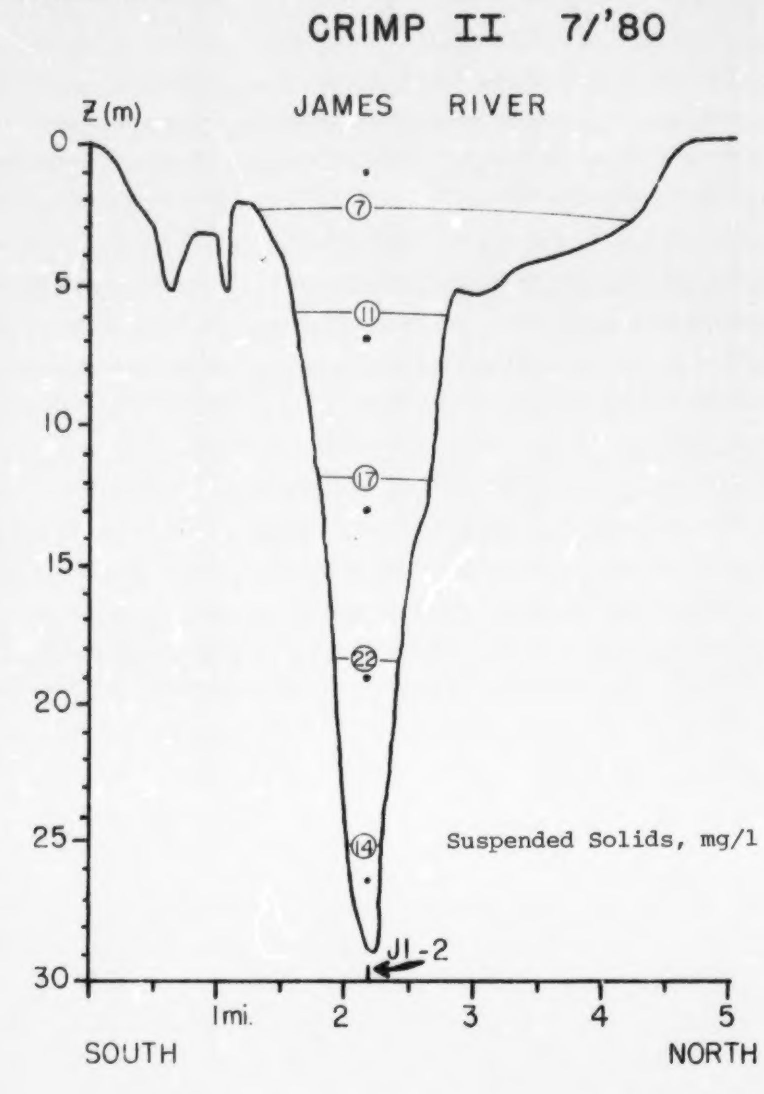
Suspended Solids, mg/1

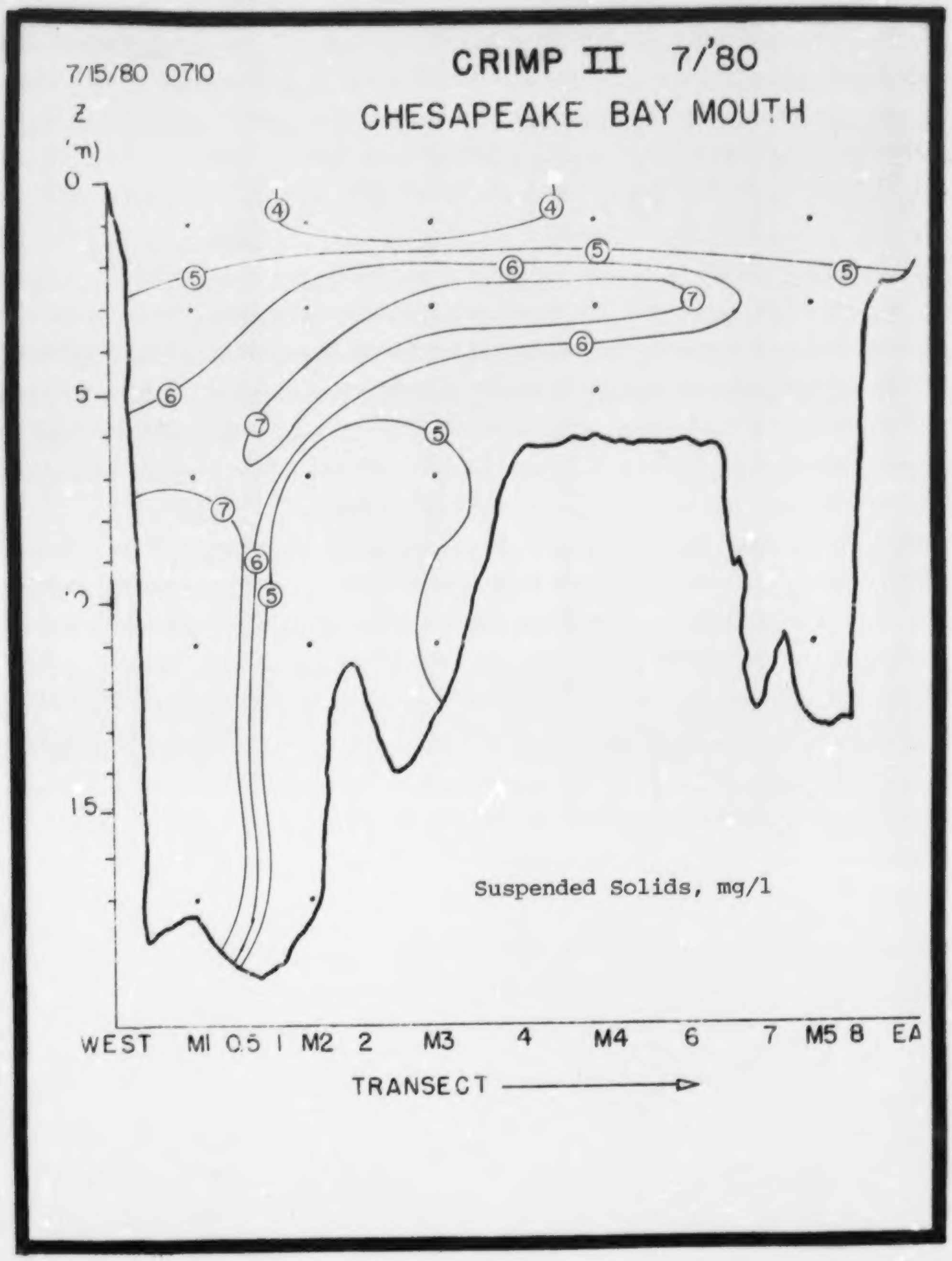

7/15/80 0618
CRIMP II 7/'80
YORK RIVER
Z (m)
0
5
10
15
0
8
8
11
14
Suspended Solids, mg/l
VRM
0
1 mi.
2
3
SOUTH
NORT

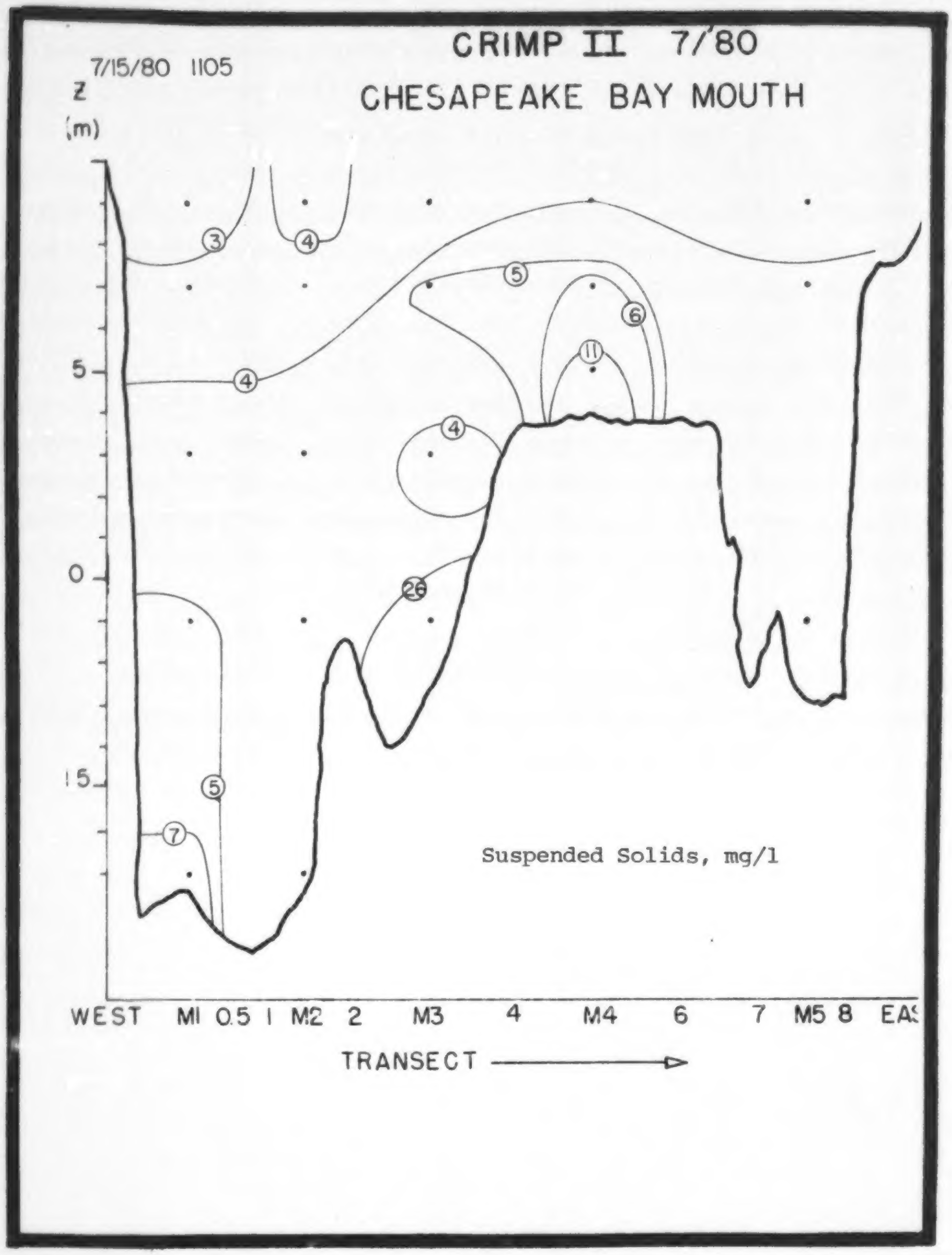

7/15/80 1640
CRIMP II 7/'80
YORK RIVER
Z (m)
0
5
10
15
0
⑩
⑩
⑮
⑪
30
Suspended Solids, mg/l
VRM
0
1 mi.
2
3
SOUTH
NORT


7/15/80 0930
CRIMP II 7/'80
JAMES RIVER
Z (m)
0
5
10
15
20
25
30
9
9±
10
40
JI-2
1mi.
2
3
4
5
SOUTH
NORTH
Suspended Solids, mg/l

7/15/80 1517

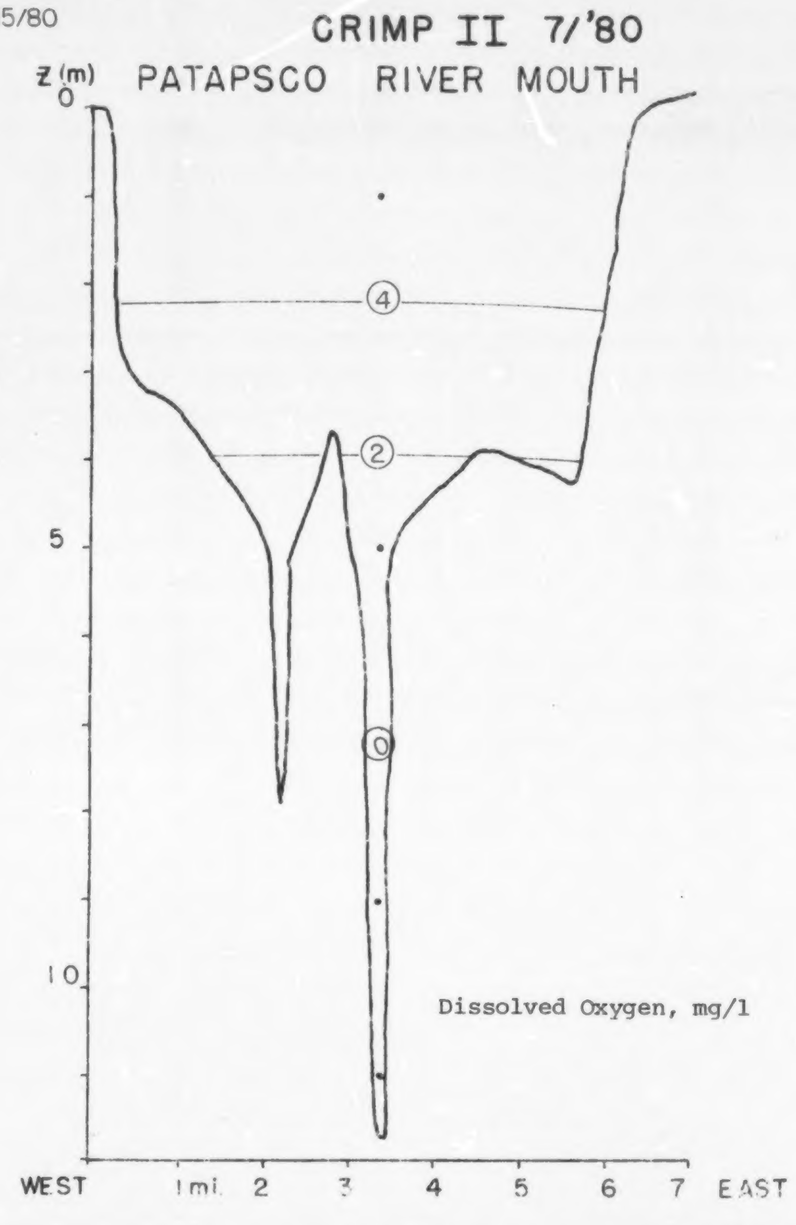

CRIMP II 7/'80
JAMES RIVER
Z (m)
0
5
10
15
20
25
30
⑦
⑪
⑰
㉒
⑭
J1-2
1mi.
2
3
4
5
SOUTH
NORTH
Suspended Solids, mg/l

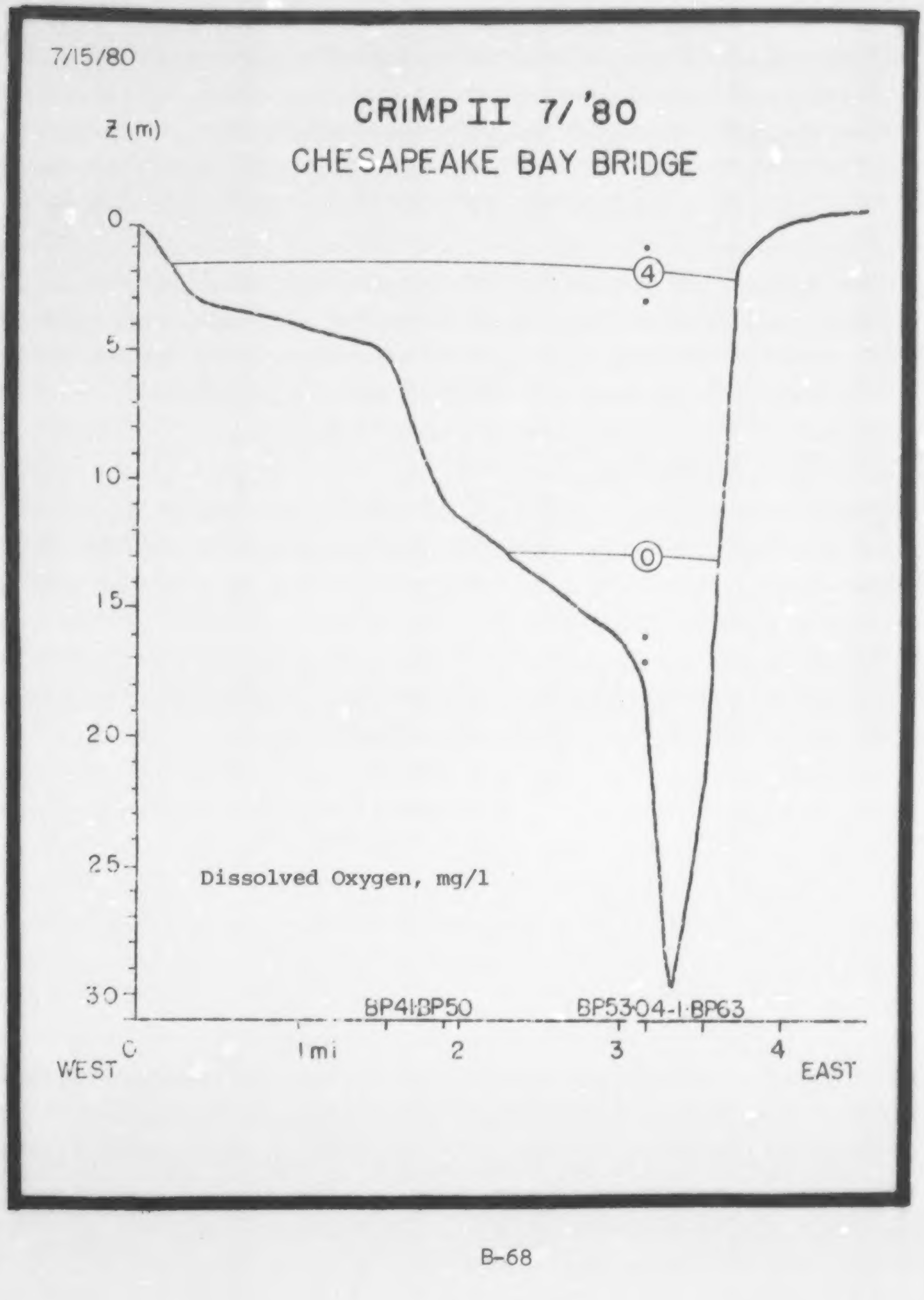

7/15/80 0710
CRIMP II 7/'80
CHESAPEAKE BAY MOUTH
Z
(m)
0
5
10
15
4
4
5
5
5
6
6
7
5
7
6
5
6
7
Suspended Solids, mg/l
WEST
M1
0.5
1
M2
2
M3
4
M4
6
7
M5
8
EA
TRANSECT

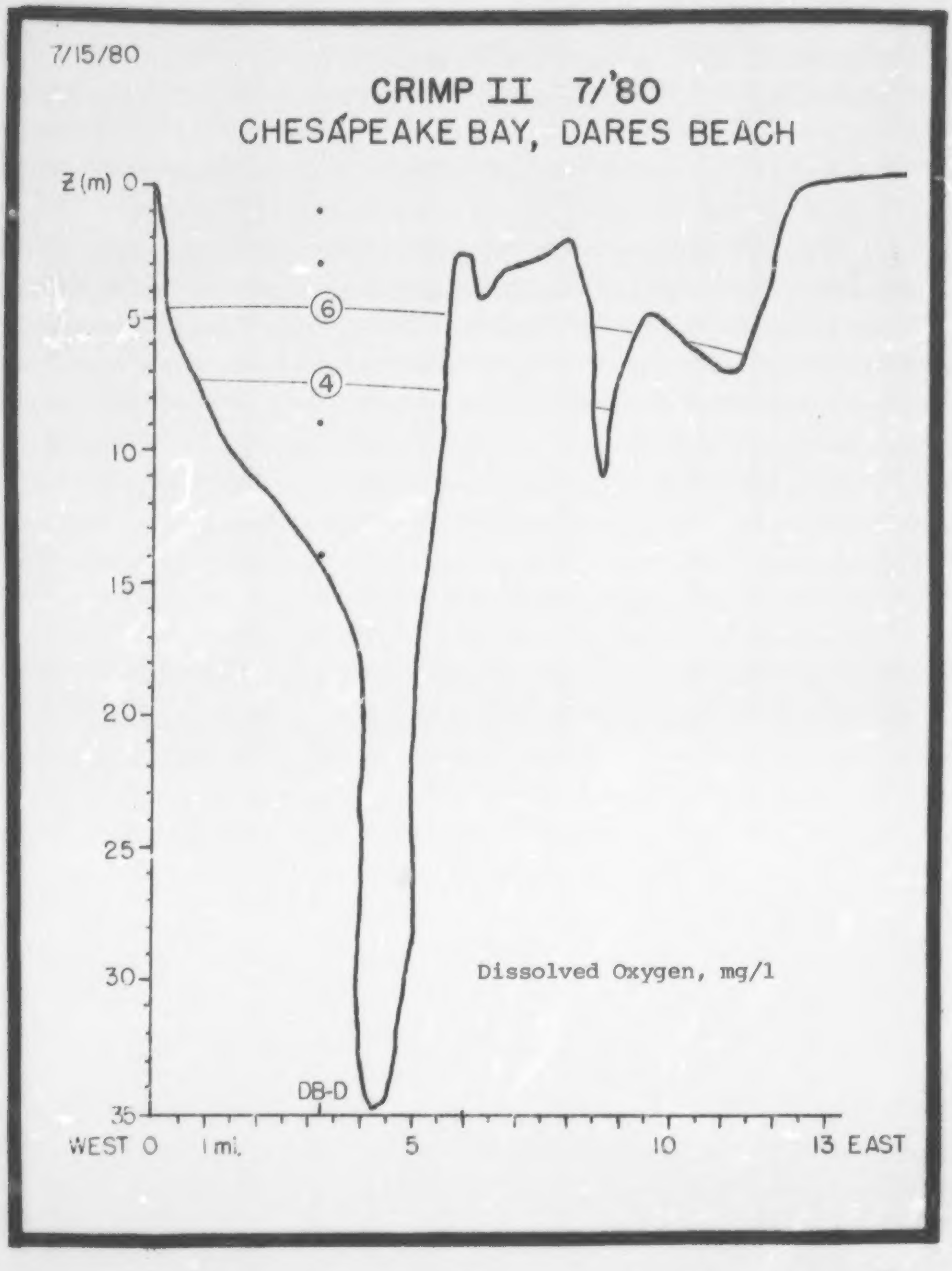

CRIMP II 7/80

CHESAPEAKE BAY MOUTH

Suspended Solids, mg/l

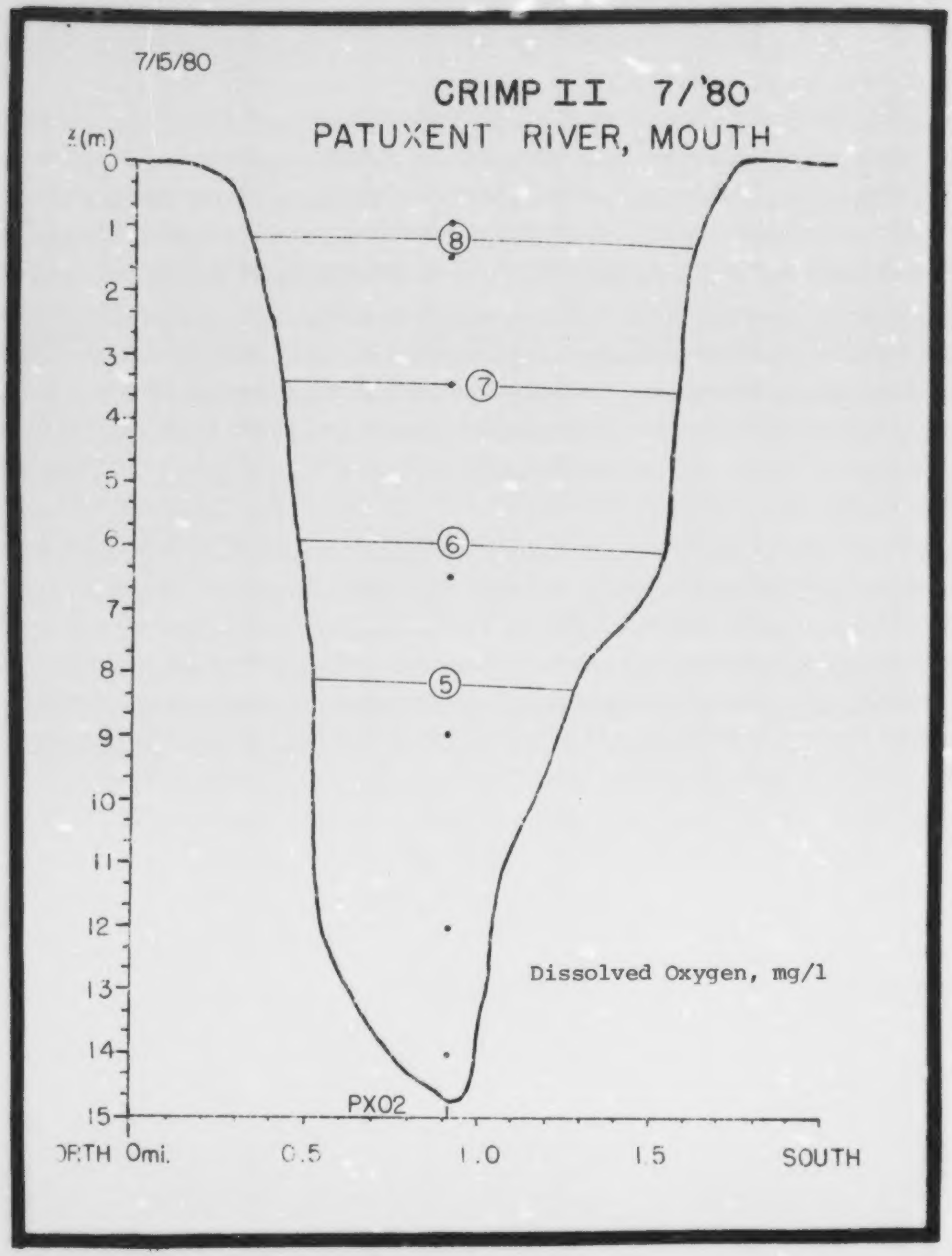

7/15/80
CRIMP II 7/'80
PATAPSCO RIVER MOUTH
z (m)
0
5
10
4
2
0
Dissolved Oxygen, mg/l
WEST
1 mi.
2
3
4
5
6
7
EAST


7/15/80
CRIMP II 7/'80
CHESAPEAKE BAY BRIDGE
Z (m)
0
5
10
15
20
25
30
4
0
Dissolved Oxygen, mg/l
BP41·BP50
BP53·04-1·BP63
WEST
0
1 mi
2
3
4
EAST



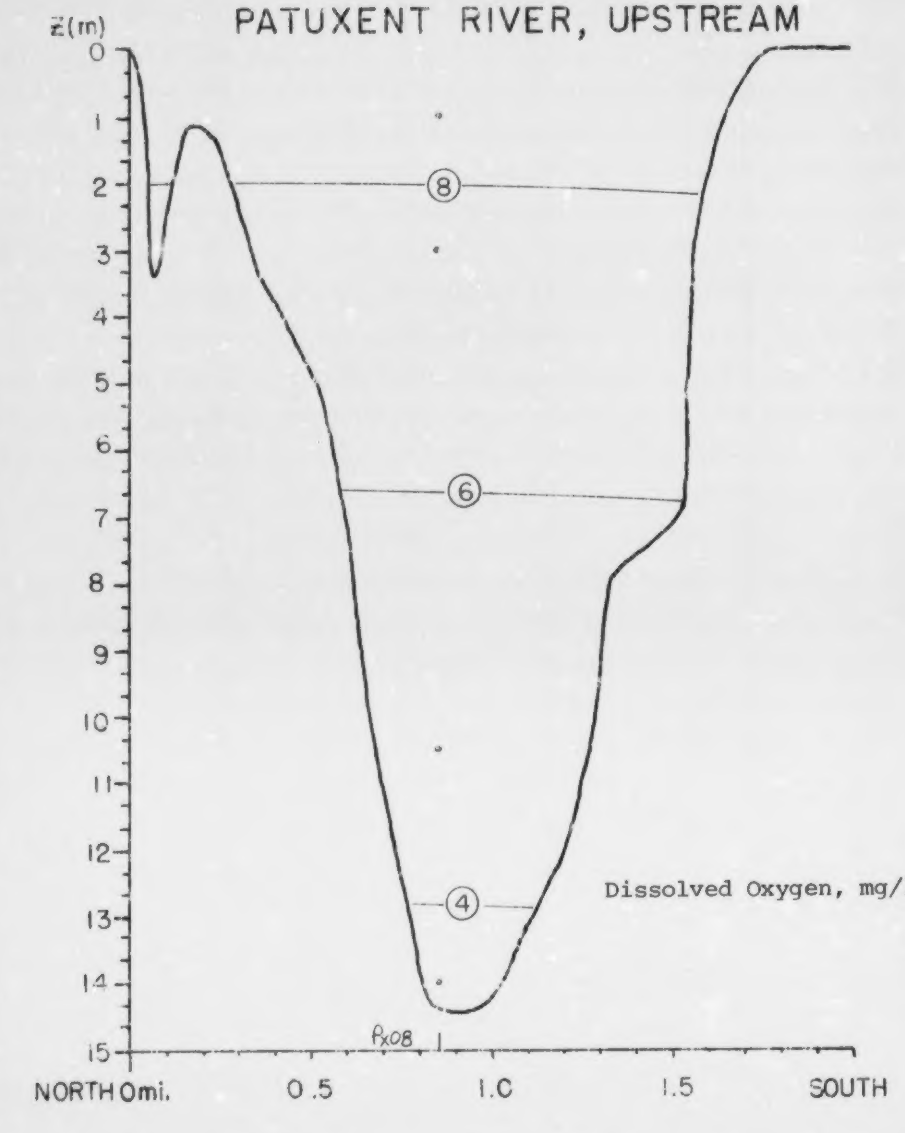
7/15/80

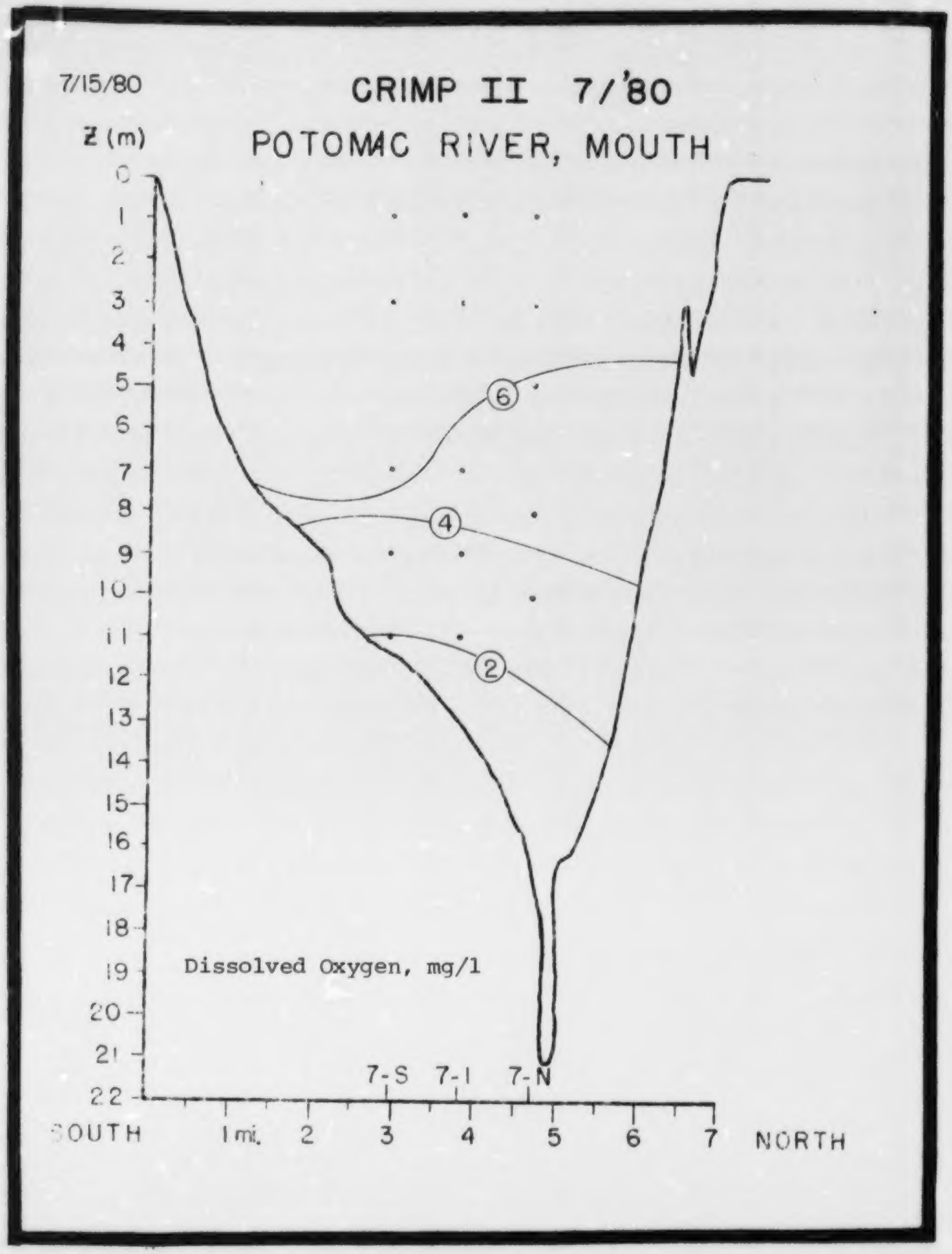
CRIMP II 7/'80
CHESAPEAKE BAY, DARES BEACH

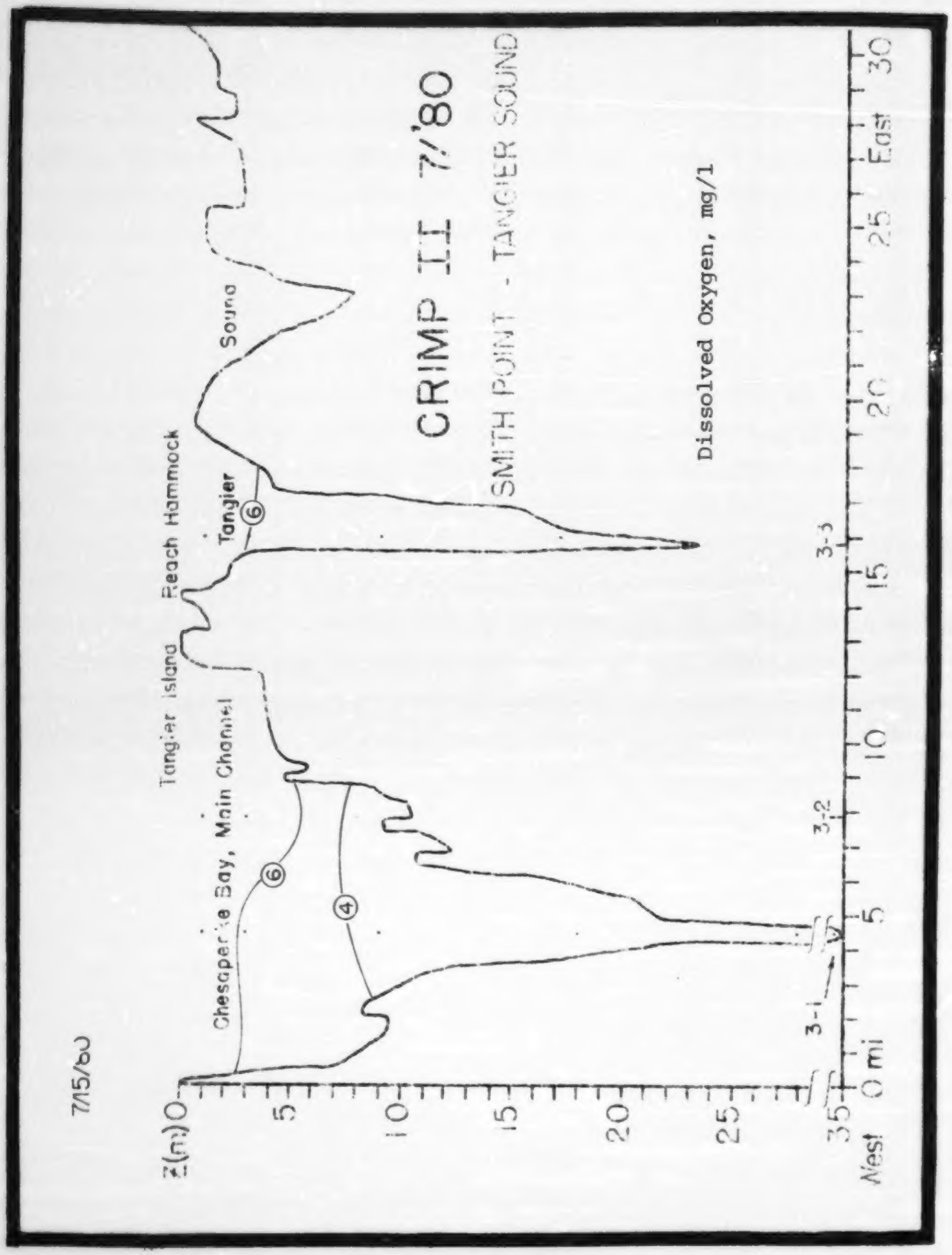

7/15/80
CRIMP II 7/'80
PATUXENT RIVER, MOUTH
Z(m)
0
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
8
7
6
5
Dissolved Oxygen, mg/l
PX02
ORTH 0mi.
0.5
1.0
1.5
SOUTH

7/15/80

CRIMP II 7/'80
PATUXENT RIVER, UPSTREAM



7/15/80
CRIMP II 7/'80
POTOMAC RIVER, MOUTH
Z (m)
0
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
6
4
2
Dissolved Oxygen, mg/l
7-S
7-1
7-N
SOUTH
1 mi.
2
3
4
5
6
7
NORTH


CRIMP II 7/'80

SMITH POINT - TANGIER SOUND

Dissolved Oxygen, mg/1

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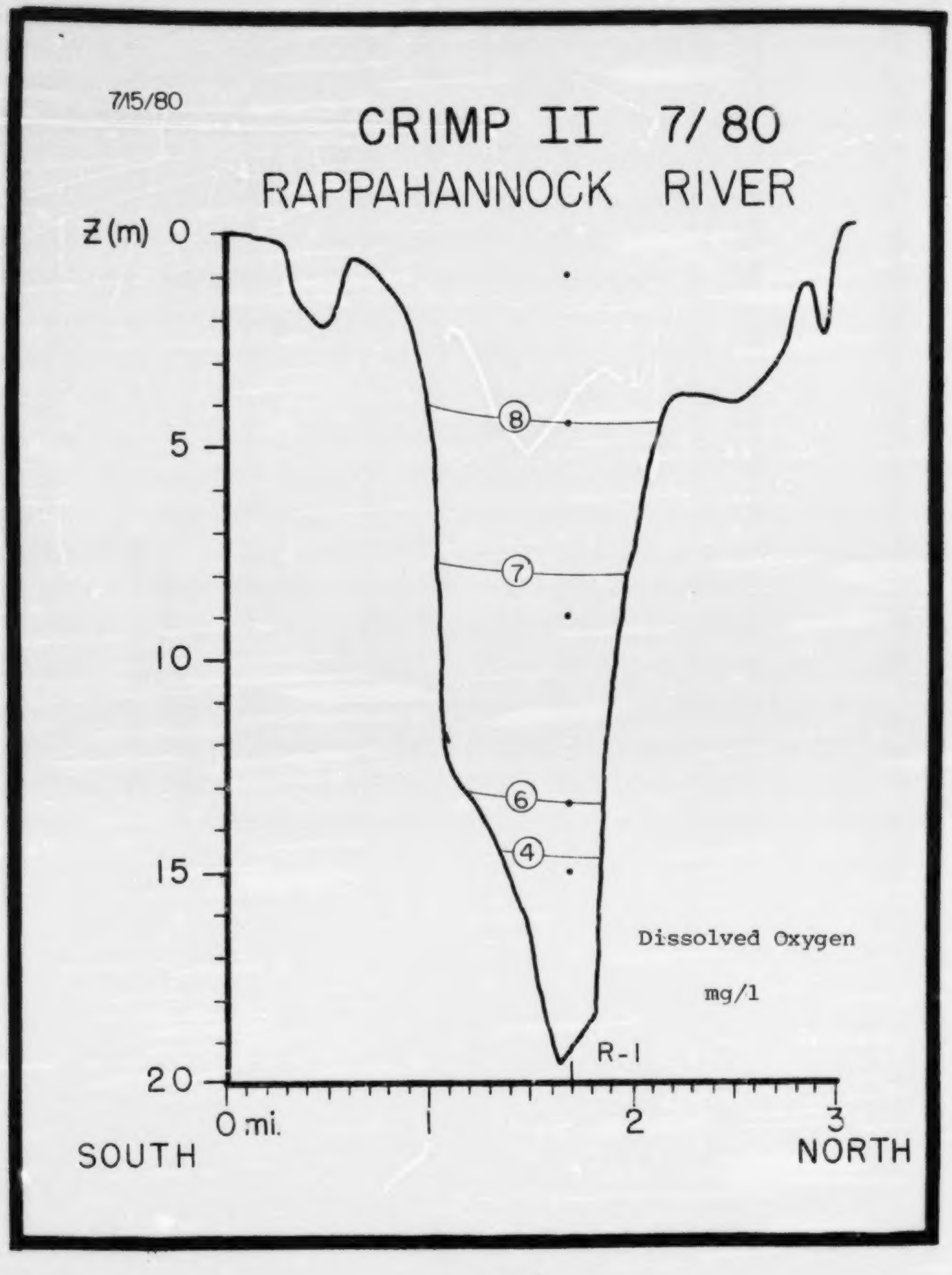
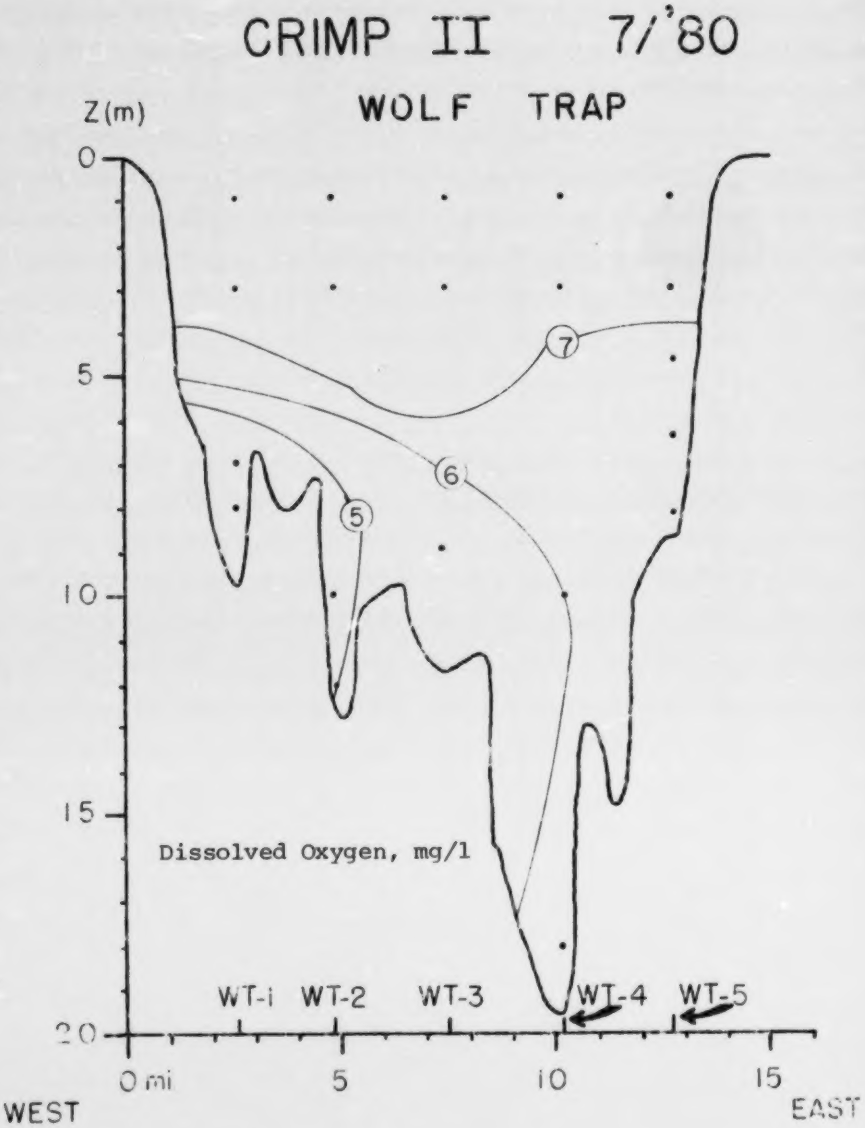
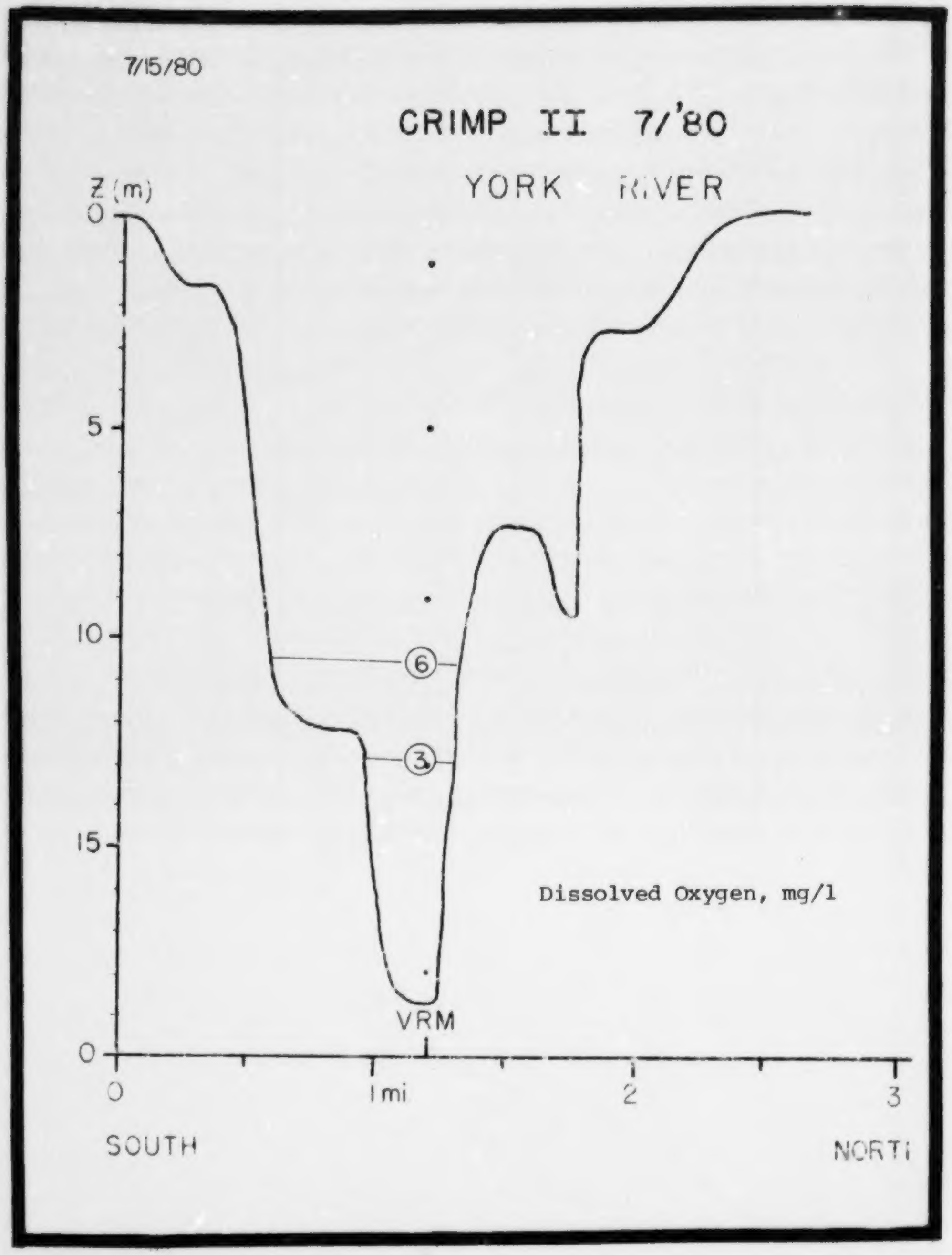
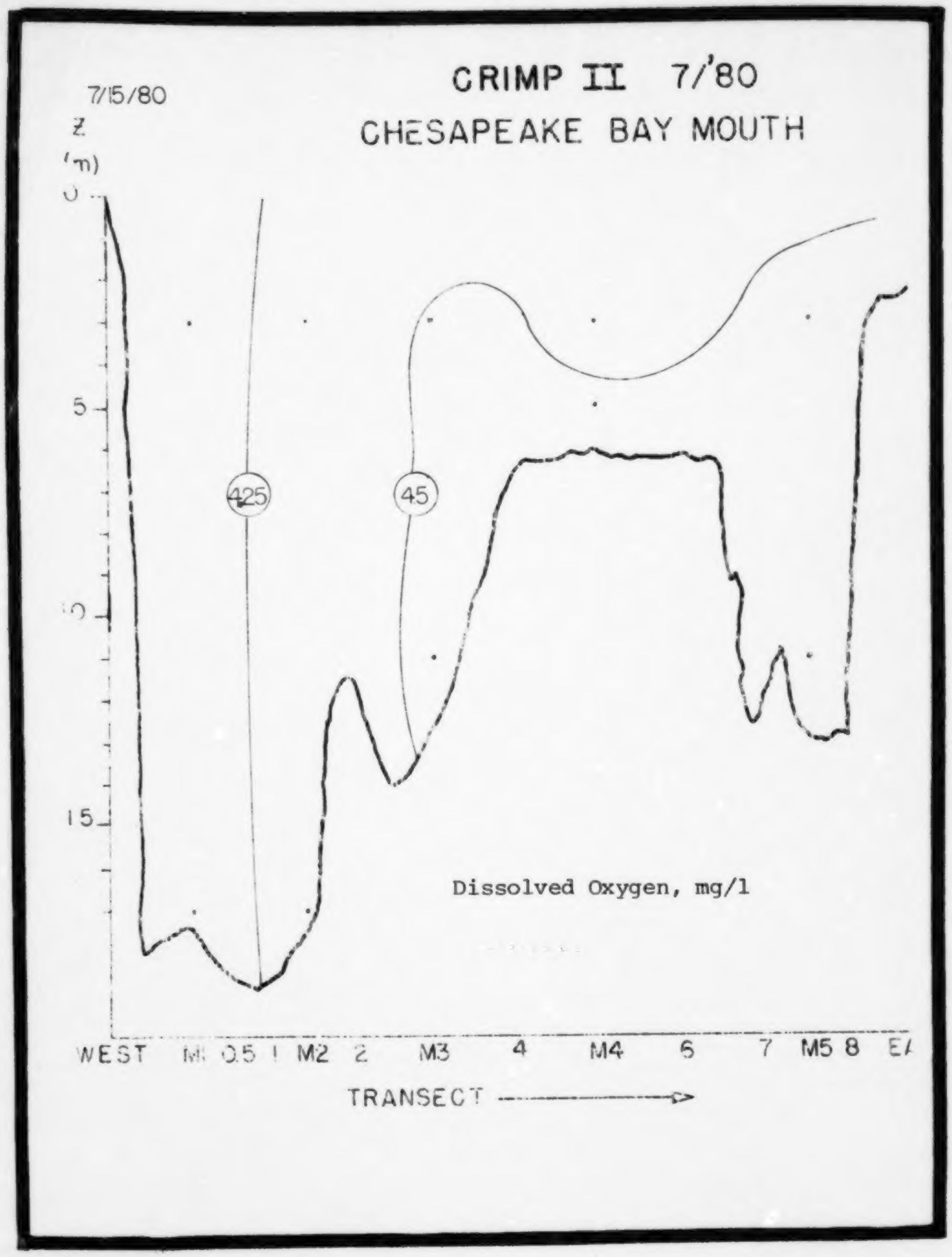

7/15/80
CRIMP II 7/80
RAPPAHANNOCK RIVER
Z(m) 0
5
10
15
20
8
7
6
4
Dissolved Oxygen
mg/l
R-1
0 mi.
1
2
3
SOUTH
NORTH

7/15/80


CRIMP II 7/'80
WOLF TRAP
Z(m)
0
5
10
15
20
7
6
5
Dissolved Oxygen, mg/l
WT-1
WT-2
WT-3
WT-4
WT-5
0 mi
5
10
15
WEST
EAST


7/15/80
CRIMP II 7/'80
YORK RIVER
Z (m)
0
5
10
15
0
6
3
VRM
0
1 mi
2
3
SOUTH
NORTH
Dissolved Oxygen, mg/l


CRIMP II 7/'80
CHESAPEAKE BAY MOUTH
7/15/80
Z
(m)
0
5
10
15
4.25
4.5
Dissolved Oxygen, mg/l
WEST
M1
0.5
1
M2
2
M3
4
M4
6
7
M5
8
E
TRANSECT →